Asymmetric Incorporation of Linoleic Acid-1-C^{14} and Stearic Acid-1-C^{14} Into Human Lymph Lecithins During Fat Absorption.* (24773)

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Hanahan and Blomstrand(1) showed that after feeding palmitic acid-1-C^{14} and oleic acid-1-C^{14} to rats, there was a difference in position of the label in lecithins of liver and intestinal tract. When palmitic acid-1-C^{14} was fed, 91-94% of the label in the lecithins of liver was localized in the beta-ester position, while, in the intestinal tract lecithins, 72-82% was found in the same position. Approximately equal labelling in alpha- and beta-ester groups was observed for lecithins of lymph. However, wtih oleic acid-1-C^{14}, nearly equal labelling was found in lecithins from all 3 different sources. Since a new technic has been developed in our laboratory(2) for cannulation of the thoracic duct in humans, it has been possible to study absorption of different labelled fats *via* the thoracic duct in man. During these studies differences in incorporation of different fatty acids into the lymph phospholipids have been observed. Furthermore an asymmetric incorporation into lymph lecithins was observed when C^{14} labelled stearic and linoleic acids were fed.

Methods. Labelled material. Stearic acid-1-C^{14} and linoleic acid-1-C^{14} were obtained from the Radiochemical Centre, Amersham, England. Labelled fatty acids were fed as free fatty acids dissolved in small amounts of olive oil. Linoleic acid-1-C^{14} was also fed as triglyceride. The labelled triglyceride was synthesized from a monoglyceride (Myverol) and equal amounts of labelled linoleic acid chloride and unlabelled linoleic acid chloride.

Procedure and sampling technic. The left thoracic duct was cannulated under local anaesthesia as described previously(2). This method permits collection of thoracic duct lymph from a given subject for 3-7 days. The investigation was performed on patients with diagnosed cancer of lungs. The patients were in good condition before and during the collection period. No intestinal disturbances were noted before or during the investigation. After the operative procedure lymph was collected at short intervals until the next morning. The labelled material was then fed mixed into the breakfast at 8 a.m. and total lymph was then collected for the following 4-6 hours. The patient was allowed to walk around the ward while on a general mixed diet. *Extraction and fractionation of lymph lipid.* The lymph was extracted with 20 volumes of 95% ethyl alcohol at room temperature and the lipids isolated after filtration, evaporation *in vacuo* at room temperature and rectified with light petroleum (B. p. 40-60°C) and made up to volume. Aliquots were taken for determination of weight, free and total cholesterol, and phosphorus(3). An aliquot was subjected to chromatography on silicic acid according to Borgström(4) and the total fat separated into cholesterol esters, neutral fat (mainly triglycerides) and phospholipids. Non-esterified fatty acids were removed by chromatography on Amberlite IRA-400 and recovered by discharging the resin with 10% HOAc in ethyl ether. Distribution of radioactivity in the different fatty acid fractions was then determined after hydrolysis and extraction of the fatty acids. Phospholipids from the major part of total fat were then isolated after precipitation with ice cold acetone and reprecipitated 3 times. The crude insoluble phospholipids obtained were then extracted with 95% ethanol, and the ethanol-soluble fraction was passed through aluminium oxide to obtain the lecithin fraction(5). The different fractions were tested with paper chromatography(6) because occasionally the aluminium technic did not remove all preformed lysolecithin from the lecithin. To obtain an absolutely pure lecithin, the "lecithin" fractions from the aluminium oxide column were pooled and subjected to chromatography on silicic acid according to Hanahan *et al.*(7).

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TABLE 1. Composition of Thoracic Duct Lymph Lipids of Man on a Mixed Diet after Adding: Exp. A, Linoleic Acid-1-C^{14} as Free Acid; Exp. B, Linoleic Acid-1-C^{14} as Triglyceride; Exp. C, Stearic Acid-1-C^{14} as Free Acid. Thoracic duct lymph was collected for the following 4-6 hours and lymph fat separated on silicic acid.

Exp.	Collection time, hr	Lymph vol, ml/hr	Total fat, g %	Cholesterol, mg % Free	Cholesterol, mg % Total	Fatty acids as % of total fatty acids: Cholesterol ester FA	Non-esterified FA	Triglyceride FA	Phospholipid FA
A	4	125	4.8	37	81	1.2	2.8	85.8	11.2
B	6	90	5.1	43	94	2.2	3.2	84.2	10.4
C	4	100	4.4	37	91	1.7	2.2	84.1	12.0

In this way pure lecithin was obtained as judged by paper chromatography. The position of the labelled fatty acids in lecithins was determined by degradation of the compound by lecithinase A into lysolecithin and free fatty acids(8). For isolation of the hydrolytic products it was suitable to use chromatography on silicic acid(7). In this way contamination with any unreacted lecithin could be avoided. The free fatty acids represented the alpha-ester position and the lysolecithin the beta-ester group. The different fractions were also tested with paper chromatography. Radioactive measurements of the various fractions were accomplished by assay of directly plated samples in a conventional gas-flow counter. All samples were counted for at least 1000 counts.

Results. The percentage of fat administered, recovered in the thoracic duct lymph fat, varies greatly (Table I), but this might be in part attributable to the rather short collection period of only 4-6 hours. Distribution of lymph fat fatty acids is in these types of experiments about the same: about 84% of lymph fatty acids are triglyceride fatty acids, 11% phospholipid fatty acids and 2% cholesterolester fatty acids, and about 3% non-esterified fatty acids.

The amount of total cholesterol in thoracic duct lymph, on a general mixed diet, varies somewhat, but can be estimated to be about 1.5-2.5 g/24 hours assuming an average lymph flow of 2500 ml/24 hours. Esterified cholesterol constitutes about 55-60% of total cholesterol. The amount of circulating cholesterol in the thoracic duct depends to a certain degree on amount of fat fed(9).

Distribution of labelled fatty acids in the various fractions of lymph lipids is shown in Table II. In Exp. A and B 90-92% of isotopically labelled acids was recovered in the triglyceride fraction, 4-6% in phospholipids, 1-2% in cholesterol ester fatty acids and 1-2% in non-esterified fatty acids.

In Exp. C there is a significant difference as to incorporation of labelled stearic acid into lymph lipids. The most significant difference is a higher incorporation into the phospholipid fatty acids, and a corresponding less incorporation into the triglyceride. Otherwise incorporation into cholesterol ester fatty acids and non-esterified fatty acids is about the same.

In all 3 experiments there is a small amount of radioactivity associated with the non-esterified fatty acids. It seems very likely that these fatty acids have been transported into the lymph from blood plasma bound to albumin(9).

In Fig. 1 the present data are compared with those obtained by Blomstrand *et al.*(9) using labelled oleic and palmitic acids. It is quite clear that oleic, palmitic and linoleic acids are handled in the same way, but that stearic acid shows a significant difference. The results conform with animal experiments of Bergström *et al.*(10,11) and Blomstrand (12).

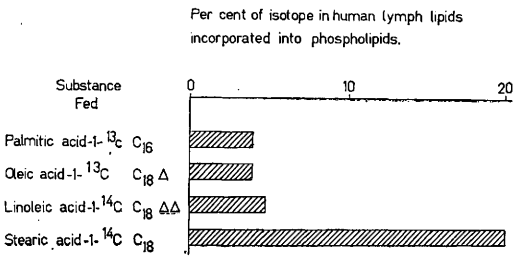

FIG. 1. Per cent of total amount of isotope in lymph lipids found associated with phospholipid fatty acids after feeding carbon-labelled fatty acids to patients with lymph fistula.

TABLE II. Distribution of Isotopic Fatty Acids in Thoracic Duct Lymph Lipids of Man on a Mixed Diet after Adding to Breakfast: Exp. A, Linoleic Acid-1-C^{14} as Free Acid; Exp. B, Linoleic Acid-1-C^{14} as Triglyceride; Exp. C, Stearic Acid-1-C^{14} as Free Acid.

Exp.	Collection time, hr	% of absorbed activity recovered in lymph lipids	% of total radioactivity in thoracic duct lymph lipids recovered as			
			Cholesterol ester FA	Triglyceride FA	Non-esterified FA	Phospholipid FA
A	4	30	1.7	91.3	4	3
B	6	35	2.0	90.0	3	5
C	4	15	1.5	75.0	3.3	20.2

In Table II are shown the results obtained on distribution of linoleic acid-1-C^{14} and stearic acid-1-C^{14} in the lymph lecithins 4-6 hours after feeding these acids to humans. Over 80% of radioactivity associated with lymph lecithins was located in the beta-ester position and the remainder was recovered in the alpha-ester group after feeding stearic acid-1-C^{14}. Although only one typical experiment is presented in Table II, data collected from 2 additional experiments give evidence for the same localization of the label in the beta-position. A significantly different distribution pattern was obtained in experiments with linoleic acid-1-C^{14}, which indicated that about 75% of radioactivity associated with lymph lecithins was located in the alpha-ester position, the remainder in the beta-ester group.

Discussion. Our data demonstrate that linoleic acid-1-C^{14} incorporated in dietary triglycerides or fed as free acid becomes esterified with the same classes of lipids in the thoracic duct lymph of man as oleic and palmitic acids(13). Evidently, digestion and absorption of these fatty acids are comparable, as well as their transfer into intestinal mucosa and resynthesis in lymph lipids. These results conform with earlier animal experiments by Bergström *et al.*(11) and Blomstrand(12).

It is of interest that stearic acid-1-C^{14} is found in a higher percentage incorporated in lymph phospholipids than was found for linoleic acid and also for palmitic and oleic acids (13). These results also extend earlier animal experiments to man(14).

From our evidence, it is obvious that there is a distinct manner in which stearic acid-1-C^{14} and linoleic acid-1-C^{14} are incorporated into thoracic duct lymph lecithins of man. This observation strongly indicates that metabolism of stearic and linoleic acids may be different, and accessibility of these acids to the enzymes concerned in incorporation into different ester linkages may play an important role. Thus it seems logical to consider that there is a metabolic difference in reactivity of the 2 ester positions of the lecithin molecule and in treatment of unsaturated and saturated fatty acids.

The final interpretation of the asymmetric incorporation of labelled fatty acids into different lecithins is difficult to make at present. It might, however, represent an important step in a different metabolism of saturated and unsaturated fatty acids. Further experi-

TABLE III. Distribution of C^{14}-Labelled Fatty Acids in Alpha and Beta Fatty Acid Position of Human Lymph Lecithins after Ingestion of Linoleic Acid-1-C^{14} and Stearic Acid-1-C^{14}. Lecithin was purified by chromatography on aluminium oxide and silicic acid and subjected to action of Lecithinase A. FFA = Free fatty acids. LL = Lysolecithin.

Exp.	Position of fatty acid	Linoleic acid-1-C^{14}		Stearic acid-1-C^{14}	
		Spec. act., cpm/mg	% of total activity	Spec. act., cpm/mg	% of total activity
A	alpha (FFA)	146	73		
	beta (LL)	54	27		
B	alpha (FFA)	225	75		
	beta (LL)	75	25		
C	alpha (FFA)			50	18
	beta (LL)			225	82

ments with different labelled fatty acids are therefore necessary to get more information, especially about the time course curves.

Summary. 1) Carboxyl-labelled linoleic and stearic acids were fed as free acids or as triglyceride esters to patients provided with thoracic duct fistula, and incorporation of these acids into various lipid classes of lymph was followed. 2) Linoleic acid was transported and incorporated into cholesterol esters, triglycerides, phospholipids and non-esterified fatty acids in the same manner as oleic and palmitic acids. Chyle triglycerides constitute the main transport form of both oleic, palmitic, linoleic and stearic acids in man. 3) When linoleic acid was fed, only about 4% of total lymph radioactivity was obtained in the phospholipids, but after feeding stearic acid about 20% of the activity was located in lymph phospholipids. 4) After isolation of lymph lecithins, there was a difference in position of the label in lecithins of lymph according to the fatty acid used. 5) After feeding linoleic acid-1-C^{14}, approximately 75% of the label in lymph lecithins was localized in the alpha-position. However, with stearic acid-1-C^{14} about 80% of the label was found in the beta-position.

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Influence of Thyroidectomy on Experimental Ascites.* (24774)

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Production of experimental canine ascites is characteristically accompanied by derangements in water and electrolyte metabolism. An important factor in this sodium and water retention by the kidney appears to be an increased level of circulating adrenocortical salt retaining hormone, presumably aldosterone (1). Previous studies reported the salutary effect of adrenalectomy and hypophysectomy on experimental ascites(2,3,4). Since functional inter-relationships are known to exist between thyroid gland and adrenal cortex(5), the following study was undertaken to evaluate the effect of thyroidectomy on ascites formation.

Materials and methods. Ascites was created in 10 mongrel dogs, weighing 10-15 kg, by partial constriction of the thoracic inferior vena cava immediately above diaphragm by a band of polyethylene tubing(6). The at-

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