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Fatty Acid Synthesis in Human Lymphocytes

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Lipid synthesis in human lymphocytes has been studied by incubation of whole thoracic duct lymph with acetate-1-^{14}C.

Total lipids were extracted from the lymphocytes and separated into cholesterol esters, triglycerides, and phospholipids, and assayed for radioactivity. Acetate-1-^{14}C was incorporated mainly into fatty acids of phospholipids and triglycerides but only to a limited extent into cholesterol esters.

With the aid of gas radiochromatography the distribution of mass and radioactivity in the fatty acids of the lymphocytes was determined.

In both triglycerides and phospholipids the major part of the radioactivity was found in fatty acids with 20–22 carbon atoms. Acetate-1-^{14}C was found to be incorporated mainly by chain-elongation of preexisting fatty acids, although *de novo* synthesis also seems to occur.

For an understanding of the lymphocyte function a better knowledge of the metabolism of these cells is essential. It has been reported that acetate-1-^{14}C incubated with human whole blood *in vitro* is incorporated into lipids.[1,2] Whole blood contains, however, a mixture of varying cellular elements with great differences in their morphological and biochemical characteristics. Recently it has also been shown by several investigators,[3-6] that human leucocytes *in vitro* are able to synthesize long chain fatty acids from acetate-1-^{14}C. Human thoracic duct lymph contains mainly lymphocytes and is almost free of platelets and leucocytes.[7] It is thus possible to study the lipid biosynthesis of the lymphocytes without interference from other active cells. A small amount of erythrocytes might occur in the lymph, but human erythrocyte lipid synthesis is very limited and probably occurs only in the youngest circulating cells.[4]

The present study demonstrates that normal human lymphocytes incorporate acetate-1-^{14}C mainly into long-chain fatty acids of phospholipids and triglycerides. The composition of the fatty acids and the radioactive pattern formed is described, after analysis with gas radiochromatography.[8] The appearance and distribution of the radioactivity indicates that the major part of the labelled fatty acids are synthesized by chain elongation.

EXPERIMENTAL

Human thoracic duct lymph was obtained in the experiments described by Blomstrand, Gürtler and Werner [9] after cannulation of the thoracic duct with a polyethylene tubing. In bottles containing 100 ml ACD-solution (1.32 % sodium acetate, 0.48 % citric acid hydrate, 1.4 % dextrose) 400 ml of human thoracic duct lymph was collected while the patient was in a fasting state. The lymph was collected directly into a bottle kept in an icebox at +4°C. The lymph obtained in a typical experiment contained 5000 lymphocytes per mm^3; it was completely free of platelets, and contained only 4—6 red cells and 1—2 polynuclear leucocytes per 100 lymphocytes.

To the lymph was added 500 μC of acetate-1-^{14}C (specific activity 56.8 μC/mole); and incubation took place at 37° in a shaking water bath for 2 h. During the incubation the lymph was flushed with 95 % oxygen and 5 % CO_2. Following the incubation the lymphocytes were isolated by centrifugation and washed with saline three times.

Lipid extraction and analysis. Extraction of total lipids from the lymphocytes was carried out with chloroform-methanol (2:1 v/v) mainly by the method of Folch, Leas and Sloane Stanley.[10] The total lipid extract was dissolved in benzene and stored under nitrogen at −15°C.

Samples of the benzene solution were taken for determination of lipid weight with a micro-Cahn balance and for total cholesterol, lipid phosphorus, and triglyceride glycerol, as described previously.[11]

Silicic acid chromatography was employed to separate the total lipids into cholesterol esters, glycerides, and phospholipid fractions.[11] The purity of the fractions was tested with thin-layer chromatography.

After chromatography portions of the various fractions were taken for determination of weight on a micro-Cahn balance. The total recovery of radioactivity after column chromatography was in the range of 90 to 95 %.

Thin-layer chromatography was used in some cases for preparation of sufficient material for gas-liquid chromatography. Thin-layer plates of silica gel were developed by ascending solvent in tanks lined by absorbant paper. For separation of total lipids into cholesterol esters, triglycerides, and phospholipids, light petroleum (b.p. 40 to 60°C)-diethylether-acetic acid 90:10:1 (v/v) was used. For phospholipid separation the chromatogram was developed in chloroform-methanol-water 65:30:5 (v/v).

After spraying one segment of the plates with rhodamine 6 G, the position of the lipids was located under ultraviolet light. The areas of silica gel containing the lipids were scraped off with a spatula, and the gel was extracted with chloroform or, in the case of phospholipids with methanol. This material was used for preparation of the methyl esters of the fatty acids and for determination of the radioactivity in the different lipid classes.

Gas-liquid chromatography: Methyl esters were formed by transesterification with 1 % sulphuric acid in benzene-methanol 1:2 (v/v). Separate mass analyses were also performed with an analytical Perkin-Elmer 801 gas chromatograph (flame ionisation detector) using a 4 m glass column with 1.2 % EGSS-X on Celite, to check the analysis of the fatty acid methyl esters.

For simultaneous determination of mass and radioactivity, a preparative Pye argon chromatograph was used as described by Blomstrand and Gürtler,[8] equipped with a strontium detector for mass and a 10 ml internal flow proportional counter for detection of radioactivity. A glass splitter was used to split the effluent gas stream and the fatty acid methyl esters were combusted to $^{14}CO_2$ as described earlier. The details of the calibration of the apparatus and columns used have been given previously.[8] The areas were measured by cutting peaks from the recorder chart and weighing the paper.

For decarboxylation of the small amounts of labelled fatty acids available, a modified procedure [12] using the Schmidt reaction was developed. The reaction vessel had a volume of 0.7 ml; 0.5—1.0 mg sodium azide was introduced into the reaction vessel. The sample to be decarboxylated, containing 0.5—1.5 mg of fatty acid, was dissolved in 0.1 ml dry benzene. A continuous stream of argon was passed through the apparatus, 10 μl conc. sulphuric acid was added, and the apparatus then was placed in a water bath at 45°C, and subjected to ultrasonic treatment. The effluent gas was bubbled through a trap with 0.5 ml ethanolamine and 1.0 ml ethyleneglycol monomethylether. The radioactivity was measured in a Packard liquid spectrometer and the average yield was found to be 85 %.

Table 1. Composition and distribution of radioactivity among major lipid classes of lymphocytes after incubation with acetate-1-^{14}C.

Patient	Per cents of lymphocyte lipid found as			Per cent of lymphocyte fat radioactivity found in		
	Cholesterol esters	Glycerides	Phospholipids	Cholesterol ester fatty acids	Glyceride fatty acids	Phospholipid fatty acids
1	0.7	17.9	81.4	2.1	30.9	67.0
2	0.5	17.6	81.9	1.8	24.5	73.7

Hydrogenation was carried out at atmosperic pressure in ethyl acetate in the presence of platinum oxide catalyst at 20°C.

The radioassay of lipid fractions other than the gas-liquid chromatography effluents was performed by liquid scintillation counting with a Packard scintillation spectrometer. Samples were counted in 15 ml of a solution of 0.6 % 2,5-diphenyloxazole and 0.03 % 1,4-bis-2-(5-phenyloxazolyl)benzene in toluene.

RESULTS

The composition and distribution of radioactivity among the cholesterol esters, triglycerides, and phospholipids of human lymphocytes after incubation with acetate-1-^{14}C is shown in Table 1.

As observed in Table 1, approximately 81 % of the lipids were identified as phospholipids (lipid phosphorus × 25) and the remaining 19 % as neutral lipids. These were composed primarily of cholesterol esters, cholesterol, triglycerides, and small amounts of titratable acids. Free fatty acids are present in low concentration in native lymphocytes. Because of the small amount of material available further experiments have to be carried out in order to elucidate the significance of the free fatty acid fraction of the lymphocytes. The phospholipids constitute the major components of the total lipids in the lymphocytes and about 70 % of the total radioactivity incorporated was recovered in the phospholipids.

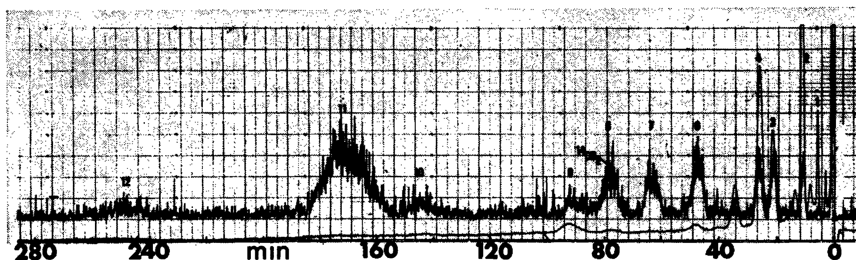

Fig. 1. Analysis of mass (lower curve) and radioactivity $^{14}CO_2$ (upper curve) of fatty acids of triglycerides in human lymphocytes after incubation with acetate-1-^{14}C. Photograph of the original recorder tracing. Polyethylene glycol succinate stationary phase at 160°C. An ionization chamber was used for determination of the mass and a flow proportional counter for determination of $^{14}CO_2$.

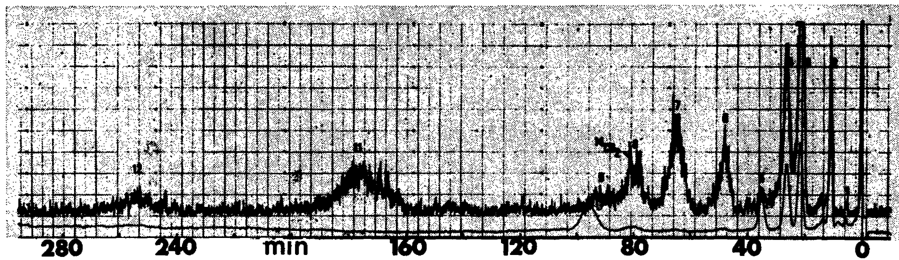

Fig. 2. Analysis of mass and radioactivity of fatty acids of phospholipids in human lymphocytes after incubation with acetate-1-^{14}C. Conditions same as in Fig. 1. 1. 14:0, 2. 16:0, 3. 18:0, 4. 18:1, 5. 18:2, 6. 20:0, 7–8. C_{20} unsaturated. 9–11. C_{22}-unsaturated. 12. C_{24}-unsaturated.

The major amount of the total radioactivity in the phospholipids was recovered in the fatty acids of the lymphocyte lecithins.

Photographs of the original recorder tracings of fatty acids in triglycerides and phospholipids are shown in Figs. 1 and 2. The fatty acid composition as well as the distribution of the radioactivity is given in these figures. In Table 2 the distribution of mass and radioactivity in the fatty acids of triglycerides and phospholipids is given. The results shown in Table 2 indicate that the fatty acid composition of the triglycerides and phospholipids of human lymphocytes is different, with a higher concentration of arachidonic acid in the

Table 2. Distribution of mass and radioactivity among the fatty acids in triglycerides and phospholipids of lymphocytes of human thoracic duct lymph after incubation with acetate-1-^{14}C. The thoracic duct lymph contained 5 × 10^3 lymphocytes per mm^3 and the radioactive substrate was added in tracer amounts. The mixture was flushed with 96 % O_2 and 5 % CO_2 under shaking at 37°C for 2 h. Values are expressed as percentage of total fatty acid methyl esters and percentage of total radioactivity. TGFA = triglyceride fatty acids, PLFA = phospholipid fatty acids. 22:a, 22:b, 24:c denotes unsaturated fatty acids not completely identified.

Fatty acid	Mass % distribution		Radioactivity % distribution	
	TGFA	PLFA	TGFA	PLFA
12:0	1.1			
14:0	7.1	1.1	0.5	0.3
16:0	24.7	20.5	2.5	4.2
16:1	5.3	2.8		
18:0	13.7	16.3	4.7	17.3
18:1	19.1	17.1	5.0	12.7
18:2	9.0	9.3	1.0	1.8
20:0	2.2	0.9	8.6	8.2
20:1	0.5	1.2	7.4	13.8
20:2	0.9	2.4	9.7	9.2
20:4	3.7	18.4		
22:0	traces	traces	4.5	4.3
22:a	traces	traces	5.4	
22:b	traces	traces	45.5	21.7
24:c	traces	traces	5.2	6.5

lymphocyte phospholipids. Radioactivity is found in a wide variety of even and odd chain, saturated and unsaturated, fatty acids in both triglycerides and phospholipids. However, radioactivity in the odd chain fatty acids accounts only for a few per cent of total radioactivity in the fatty acids. A comparison of the distribution pattern of the radioactivity in the triglycerides and phospholipids (Figs. 1 and 2) indicates that there are two distinct different radioactivity distribution patterns in these two lipid classes.

A comparison of the data in Table 2 on the basis of percentage of total radioactivity in the various groups of fatty acids indicate that significant decrease in percentage of radioactivity in both 14:0 and 16:0 with a significant increase in percentage of radioactivity in saturated and unsaturated C_{20} fatty acids and fatty acids with retention time corresponding to 22 carbon atoms and greater. These findings are qualitatively similar in both triglycerides and phospholipids fatty acids of the lymphocytes, although there are quantitative differences. In both cases there is, however, a tendency to a concentration of the major portion of the total radioactivity in a few fatty acids with 20—24 carbon atoms.

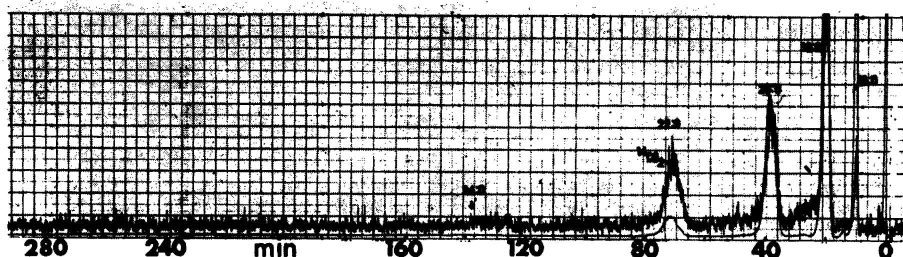

Fig. 3. Analysis of mass and radioactivity of fatty acids in lymphocyte phospholipids after hydrogenation. Conditions same as in Fig. 1.

Table 3. Distribution of radioactivity in the fatty acids of phospholipids of human phospholipids, before and after hydrogenation.

Fatty acid	Radioactivity % distribution before hydrogenation	Radioactivity % distribution after hydrogenation	Per cent radioactivity in the carboxyl group.
14:0	0.3		
16:0	4.2	5.8	68
18:0	17.3	34.3	74
18:1	12.7		
18:2	1.8		
20:0	8.2	29.4	90
20:1	13.8		
20:2	9.2		
22:0	4.3	24.7	79
22:a			
22:b	21.7		
24:0		5.8	74
24:c	6.5		

A portion of the phospholipid fatty acids of the lymphocytes was hydrogenated and subjected to analyses of mass and radioactivity, as shown in Fig. 3. The radioactivity is then concentrated in 16:0, 18:0, 20:0, and 22:0 fatty acids with the major amount of radioactivity in fatty acids with 20 and 22 carbon atoms (Table 3). After preparative gas chromatography the different saturated fatty acids were decarboxylated (Table 3). The results of the decarboxylation indicate that in all of the saturated fatty acids analyzed, more than 60 % of the radioactivity was recovered in the carboxyl group. Considerable quantities of acetate-1-^{14}C were incorporated into C_{22} polyunsaturated fatty acid; radioactivity was also recovered in the carboxyl group of a fatty acid 24 carbon atoms, which indicates the presence in human lymphocytes of a system capable of forming very long chain fatty acids.

DISCUSSION

The study indicates that lipid synthesis occurs in lymphocytes of human thoracic duct lymph. The present observation that human lymphocytes actively synthesize lipids is in accord with recent reports,[3-6] that normal leucocytes synthesize lipids. The lymphocytes of human thoracic duct lymph provide a very suitable model for the study of lipid metabolism, because they can be obtained without significant contamination of other actively lipid synthesizing cells.

From the present investigation it is apparent that acetate-1-^{14}C is incorporated mainly into the phospholipids of the lymphocytes. Accumulating evidence points to the immunologic role of the lymphocytes in the formation and carriage of antibodies.[13] Furthermore it has been reported that phospholipids might be essential for serologic reactions.[14] Further studies are thus indicated to elucidate the phospholipid synthesis in human lymphocytes. The results obtained are in contrast to those of Hennes and coworkers on human trombocytes.[15,16] These investigators found that the fatty acids of neutral lipids contained more radioactivity than the phospholipids after incubation with acetate-1-^{14}C.

As is apparent from Figs. 1 and 2 the pattern of fatty acid radioactivity in phospholipids and triglycerides was distinct. In both lipid classes there is a clear tendency toward synthesis of fatty acids with 20—24 carbon atoms. The differences in the incorporation of certain fatty acids may be due to differences in specificity of the enzymes which are involved in the formation of triglycerides and phospholipids.

Regarding the mechanism for the fatty acid synthesis in human lymphocytes the present data do not give a full explanation. From the data in Table 3, it is apparent that after hydrogenation of the unsaturated fatty acids in the phospholipids and following decarboxylation, about the same amount of radioactivity was found in fatty acids with 18 and 20 carbon atoms (34.3 and 28.4 %, respectively). However, 90 % of the radioactivity was recovered in the carboxyl group of the fatty acids with 20 carbon atoms against 74 % in the carboxyl group in the 18 carbon atom fatty acid. The results of the decarboxylation thus indicate that the fatty acids with 20 carbon atoms are formed

mainly by chain elongation, according to the elongation mechanism described by Wakil.[17]

Since the completion of this work Miros *et al.*[18] have recently reported similar results after incubation of human leucocytes with acetate-1-^{14}C. Further analyses of the individual fatty acids will reveal to what extent both the elongation and desaturation systems contribute to the *in vitro* biosynthesis of the unsaturated fatty acids in the lymphocytes.

The results of the present investigation indicate that under the experimental conditions employed there is a specificity for the formation of C_{20} to C_{22} fatty acids. Chain elongation seems to be more important than *de novo* synthesis.

The clarification of the significance of the synthesis of mainly higher fatty acids of phospholipids in human lymphocytes in relation to the function of the lymphocytes *in vivo* under physiological and pathological circumstances remains for speculation and further study.

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