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Lipid Biosynthesis in Human Thoracic Duct Lymphocytes and Thymocytes

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The biosynthesis of lipids in human thymocytes (thymus lymphocytes) and thoracic duct lymphocytes *in vitro* was studied by incubation of the cells with acetate-1-^{14}C.

The label was mainly incorporated into cholesterol and the fatty acids of glycerides and phospholipids. Among the phospholipids, radioactivity was found mainly incorporated into lecithin. In glycerides, phospholipids and individual phospholipids, the main radioactivity was confined to fatty acids with 18 – 24 carbon atoms, indicating that the label was incorporated mainly by chain elongation. An exception, however, was thymocyte lecithin where 50 % of the radioactivity was confined to palmitic acid.

Although there are slight differences between the synthesis of different lipid fractions of human thymocytes and thoracic duct lymphocytes, the principal impression is the striking similarity indicating a similar biochemical mechanism for synthetizing lipids.

Although the lymphocytes in blood, lymph, spleen, bone marrow, lymph nodes, and thymus are morphologically almost identical, variations in their functional capacity, responses to antigens, life span, cellular and/or tissue origin and their pathway of circulation are generally recognized. In view of the previous finding that there is an efficient biosynthesis of various lipids by human thoracic duct lymphocytes,[1] it appeared to be of interest to study the possibility that there are differences in the biosynthesis of the lipids by human thymocytes (thymus lymphocytes) and thoracic duct lymphocytes. In the present work, the lipid composition and incorporation of ^{14}C-acetate into different lipid fractions, inclusive individual fatty acids in human thoracic duct lymphocytes and thymocytes have been studied. In previous work there is limited information concerning composition of different fatty acids in the different lipid fractions in human lymphocytes and nothing has been previously reported concerning lipid composition in human thymocytes.

EXPERIMENTAL

The biological material, *i.e.* lymph and thymi, used for the study were obtained from patients operated on for diagnostic and/or therapeutic reasons. The material had otherwise not been used.

Patient codes are given as a number and the letter L if lymph was obtained, T if thymus was obtained. The experiment codes are the same as the patient codes or if many experiments were done with lymph from the same patient a number is added after the letter.

Clinical. Thoracic duct lymph for 10 experiments was obtained from 7 patients. More important clinical data on the patients is found in Table 1a.

Table 1a. Patients from whom thoracic duct lymph was obtained.

Patient	Sex	Age	Diagnosis
3/L	♂	69	Suspicion of gastric carcinoma [a]
23/L	♀	52	Mammary carcinoma
45/L	♀	48	Adispositas dolorosa
46/L	♀	31	Glomerulonephritis and uraemia. In hemodialysis.
48/L	♂	70	Thyroid carcinoma
54/L	♂	25	Glomerulonephritis and uraemia. In hemodialysis.
55/L	♂	28	Uraemia. Nephrectomized. In hemodialysis.

[a] Was not verified by gastric resection.

In patient 3/L, 23/L, and 48/L the thoracic duct was cannulated in order to detect and drain cancer cells.[2] Patient 45/L was cannulated in order to investigate the possibility of a metabolic disorder. It was shown that the fatty acid synthesis was affected in the pathologic but not in the healthy fat tissue.[3] In patients 46/L, 54/L, and 55/L thoracic duct lymph was drained as immunosuppressive therapy prior to kidney transplantation[4,5].

Thymi were removed for surgical reasons in search for metastasis and/or the parathyroids.[6] The more important clinical data on these patients are found in Table 1b.

Table 1b. Patients from whom thymi were obtained.

Patient	Sex	Age	Diagnosis
33/T	♀	26	Thyroid carcinoma
34/T	♀	29	Thyroid carcinoma
36/T	♀	23	Toxic goitre
52/T	♀	21	Thyroid carcinoma

Cell culture procedure. Lymph was collected and incubated with acetate-1-^{14}C as earlier.[1] In experiments 3/L, 23/L, 45/L, and 55/L/4 2 IU heparin (Heparin, Vitrum) per ml lymph were used as anticoagulant instead of ACD-solution. The present results and those obtained earlier [1] did not indicate that the lipid composition and the synthesis

is different when lymphocytes are incubated in ACD-lymph in comparison with lymphocytes incubated with heparin. Lymph was collected during the first two days of drainage except in experiments 46/L/1 and 46/L/2, where the lymph was collected on the 10th and 12th day, respectively. The lymph was usually incubated directly. In experiments 46/L/1, 46/L/2, and 48/L/3 the lymph was, however, centrifuged and the main part of the lymphocytepoor supernatant was removed by suction. In experiments 54/L/3 and 54/L/6 the lymphocytes were isolated by centrifugation, washed and then incubated in Krebs balanced saline solution, medium 1 [7] (Krebs medium) and lymph, respectively. The lymphocyte viability was 90 – 100 % as judged by trypan blue exclusion.[8] The incubates contained 4 – 67 × 10^3 lymphocytes per μl and 0 – 10 erythrocytes per lymphocyte but practically no other cells as determined by microscopic investigations.[9] Acetate-1-^{14}C (specific radioactivity 53 – 57 mCi/mmol, The Radiochemical Centre, Amersham, England) was added in a concentration of 1 – 10 μC/ml incubate and the incubation time varied between 2 and 6 h. The results did not indicate that the different radioactivity dosage or incubation time influenced the results.

The thymi were, immediately after removal, minced in precooled Krebs medium. The obtained thymus suspension was filtered through gauze compresses. The filtered suspension contained 30 – 340 × 10^3 thymus lymphocytes and 1 – 18 × 10^3 erythrocytes per μl and very few other cells. The thymocyte viability was 80 – 95 %. The thymus suspensions were incubated for 2 – 5 h with acetate-1-^{14}C in a concentration of 5 – 25 μC/ml media.

All incubations took place at +37°C in a shaking water bath and the media were continuously flushed with 5 % CO_2 + 95 % O_2. After incubation lymphocytes and thymocytes were immediately isolated at +4°C.[10]

Lipid analysis. The lipids were extracted and then separated by silicic acid chromatography into three fractions, (1) hydrocarbons + cholesterol esters, (2) glycerides, free cholesterol and free fatty acids, (3) phospholipids and other polar lipids as described earlier.[10,11] The radioactivity and weights of the lipid fractions were determined.[10]

The lipid fractions were then analyzed by thin-layer chromatography (TLC).[10,12] Silica gel G plates were developed in pentane:ether, 9:1, to separate the neutral lipids and in chloroform:methanol:13 N ammonia, 14:6:1, to separate the phospholipids. The mass distribution among lipids separated by TLC was determined by transmission densitometry in a Vitatron TLD 100 densitometer (Vitatron, Dieren, Holland). A representative recording is shown in Fig. 1. The radioactivity distribution among the lipids

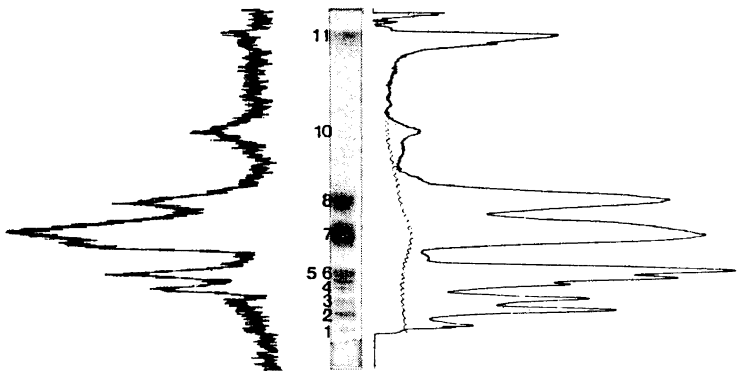

Fig. 1. Photo, radioscan (left) and densitometric recording (right) of thin-layer chromatogram with separated phospholipids from lymphocytes incubated with acetate-1-^{14}C. 1. Start fraction. 2. Fraction with the chromatographic behaviour of phosphatidic acid. 3. Fraction with the chromatographic behaviour of phosphatidyl serine. 4. Fraction with the chromatographic behaviour of phosphatidyl inositol. 5 – 6. Sphingomyelin separated into two portions. 7. Lecithin. 8. Phosphatidyl ethanolamine. 10. Fraction with the chromatographic behaviour of cerebrosides. 11. Front fraction, mainly contaminants.

separated by TLC was measured in two ways: (1) The charred lipid bands were scraped off the plates, emulsified in 15 ml Insta Gel emulsifier (Packard Instrument Co., Ill., USA) to which 1 ml distilled water had been added and counted in a liquid scintillation spectrometer (Packard Tri Carb Counter, Model 3003) with an external standard. This procedure was applied for all neutral lipids in the study. (2) The radioactivity of the separated phospholipids was measured on the plate with a Berthold thin-layer scanner II (Berthold Laboratories, Wildbad, W. Germany) equipped with a 2 π detector and connected to a ratemeter (LB 242 K). The output of the ratemeter was monitored on a Methrohm Labograph E 478 (Methrohm AG, Herisau, Switzerland). A representative recording is shown in Fig. 1.

The percentage distribution of mass and radioactivity was calculated by cutting out the peaks from copies of the densitometric and radioactivity scanning recorder charts and weighing the paper. The reproducibility of the techniques for determining mass and radioactivity distribution was found reasonably reliable.[12]

The lipids were identified by comparing their retention times on TLC with those of the purified references (see below). In some cases for further identification the lipids were isolated from the gel and rerun in another TLC system or the eluted lipids were analyzed for phosphorus content and fatty acid composition.

Fatty acid analysis. The fatty acid composition and the distribution of radioactivity among the fatty acids of different lipid fractions were analyzed by radio gas chromatography mainly in accordance with Blomstrand [1] and Blomstrand and Gürtler.[13] A Perkin-Elmer 801 gas chromatograph with flame ionization detector was used. The glass column (2.20 m long and 10 mm ID) was packed with 14 % PEGS on acid-washed and siliconized Chromosorb W (100 – 120 mesh). The fatty acid methyl esters were identified by comparing their retention times to those of reference fatty acid methyl esters (Supelco Inc., Pa., USA, or The Hormel Institute, USA). Fatty acids of the different phospholipids from thoracic duct lymphocytes of one experiment (48/L/3) have been analyzed with an improved radio gas chromatographic method using hydrocracking as described recently.[14]

Fatty acid methyl esters, which did not separate on the PEGS column, were trapped from the effluent with glass tubes (5 cm), packed with 3 % Se 30 on Chromosorb W (100 – 120 mesh). The trapped methyl esters were eluted with chloroform and identified by GLC-mass spectrometry (LKB 9000) using an Se 30 column. The conditions for the mass spectrometer were: molecular separator temperature 240°C; electron energy 70 eV; column temperature 150 – 200° with a program rate of 3°/min.

Lipid standards. Phospholipids from human brain tissue were separated according to Svennerholm.[15] The individual phospholipids were isolated from the thin-layer plates and checked on different thin-layer systems. The fatty acids of these phospholipids were also analyzed and agreed with the results published by Svennerholm.[15,16] These phospholipids were used as phospholipid standards. Individual phospholipids from bull testis were also prepared in the same way and used as reference phospholipids.

The following lipids were purchased: cholesteryl oleate, triolein, oleic acid, and methyl oleate (Mixture No. 1); cholesterol, cholesteryl oleate, triolein, oleic acid, and hydrogenated lecithin (Mixture No. 2); monopalmitin, dipalmitin, and tripalmitin (Mixture No. 3); all obtained from the Hormel Institute. Lysolecithin, sphingomyelin B-grade, phosphatidyl ethanolamine A-grade, synthetic dipalmitoyl lecithin A-grade from Calbiochem, Los Angeles, Calif., USA. Phosphatidyl serine, cerebrosides, cardiolipin, sulphatides, phosphatidyl inositol from Applied Science Laboratories, Pa., USA. Phosphatidyl choline-1,2-^{14}C (spec. radioactivity 117 mCi/mmol) and phosphatidyl ethanolamine-1,2-^{14}C (44 mCi/mmol) were purchased from Tracerlab., Mass., USA. All these lipids have been used as reference compounds after purification in appropriate TLC-systems.

RESULTS

Incorporation of acetate-1-^{14}C into lipids. The lipid composition of the lymphocytes varied slightly among the experiments but in all lymphocytes the phospholipids were consistently the largest fraction (Table 2a). The thymocytes contained a lower percentage of phospholipids than the lympho-

Table 2. In 6 experiments with lymphocytes (3/L, 45/L, 46/L/1, 48/L/1, 54/L/6, and 55/L/4) and 2 experiments with thymocytes (33/T, 52/T), the cells were incubated with acetate-1-^{14}C at 37°C. Total lipids were extracted and separated by silicic acid chromatography into three fractions: (1) hydrocarbons+cholesterolesters, (2) other neutral lipids, and (3) phospholipids. The weights and the radioactivity of the fractions were determined. Fraction 2 was further analyzed by TLC. The lipids were visualized by charring. The mass distribution among the separated lipids was estimated by densitometry. The lipid bands were then scraped off and the radioactivity determined. The distribution of mass among the lipids is given in Table 2a and the distribution of radioactivity in Table 2b.

Table 2a. Distribution of mass among lipids from lymphocytes and thymocytes. Values are expressed as relative percentages of total lipids.

Fraction [a]	Lipid	Lymphocytes						Thymocytes	
		3/L	45/L	46/L/1	48/L/1	54/L/6	55/L/4	33/T	52/T
1	Hydrocarbons+ cholesterolesters	6.2	21.4	12.9	7.4	13.0	13.0	4.0	11.2
2 a	Monoglycerides	9.6	3.0	4.0	3.5	3.6	4.5	6.5	4.4
2 b	Cholesterol [b]	12.6	4.8	6.1	5.2	7.0	6.7	6.0	6.8
2 c	Free fatty acids	3.9	1.6	1.0	3.5	1.9	2.1	Tr	0.7
2 d	Triglycerides	7.6	10.4	15.4	7.7	12.7	6.4	57.8	20.1
2 e	Unknown neutral lipids	2.0	4.0	—	0.8	1.2	2.7	—	3.0
3	Phospholipids	58.1	54.8	60.6	71.9	60.6	64.6	25.7	53.8
	Total lipids	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Numbers refer to the silicic acid and letters to the thin-layer separation.
[b] Contained also small amounts of mass and radioactivity confined to free fatty acids and diglycerides.

cytes. In the biosynthesis of lipids from acetate there was no difference between thymocytes and lymphocytes (Table 2b). Considerable differences among the experiments have been observed, however, which may depend on the different cell sources and conditions. Experiment 46/L/1 demonstrated the

'able 2b. Distribution of radioactivity among lipids from lymphocytes and thymocytes. Values are expressed as relative percentages of radioactivity in total lipids.

Fraction [a]	Lipid	Lymphocytes						Thymocytes	
		3/L	45/L	46/L/1	48/L/1	54/L/6	55/L/4	33/T	52/T
1	Hydrocarbons+ cholesterolesters	0.0	2.2	1.1	6.7	2.0	3.7	15.7	0.8
2 a	Monoglycerides [c]	14.8	12.8	18.3	8.5	12.4	14.8	14.5	22.8
2 b	Cholesterol [b]	5.4	7.1	26.3	4.6	11.7	8.0	3.0	9.5
2 c	Free fatty acids	1.3	4.8	8.3	6.2	4.3	5.8	2.9	5.2
2 d	Triglycerides	2.7	9.4	14.2	18.8	26.6	8.0	12.7	21.7
2 e	Unknown neutral lipids	1.5	3.0	1.6	2.1	1.1	3.6	1.7	4.0
3	Phospholipids	74.3	60.7	30.2	53.1	41.9	56.1	49.5	36.0
	Total lipids	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] See note [a] in Table 2a. [b] See note [b] in Table 2a. [c] Part of the radioactivity is confined to an unknown ›mpound.

Table 3. In four experiments with lymphocytes (3/L, 48/L/1, 54/L/3, and 54/L/6) and in two experiments with thymocytes (33/T and 52/T), the cells were incubated at +37°C. The lymphocytes were, with one exception (54/L/3), incubated in lymph. The thymocytes and lymphocytes of experiment 54/L/3 were incubated in Krebs-medium. Acetate-1-^{14}C was added at start of incubation. The phospholipids were isolated by silicic acid chromatography. The phospholipids were further separated by thin-layer chromatography. The radioactivity distribution among the separated phospholipids were determined by radioactivity scanning and, after charring, the mass distribution was estimated by transmission densitometry. The distribution of mass among the phospholipids is given in Table 3a, and the distribution of radioactivity in Table 3b.

Table 3a. Mass distribution among phospholipids of lymphocytes and thymocytes. Values are expressed as percentages of total mass of phospholipids.

TLC fraction	Phospholipid	Lymphocytes 3/L	48/L/1	54/L/3	54/L/6	Thymocytes 33/T	52/T
1	Start fraction [a]	4.9	1.3	2.1	1.6	6.2	2.0
2	Phosphatidic acid	3.7	2.2	7.6	7.5		3.9
3	Phosphatidyl serine	2.5	7.8	6.6	4.1	6.4	5.3
4	Phosphatidyl inositol	9.0	8.6	4.9	4.1	6.9	8.1
5–6	Sphingomyelin	13.6	12.4	20.2	25.2	7.4	9.9
7	Lecithin	51.0	39.3	30.0	30.9	42.2	41.4
8	Phosphatidyl ethanolamine	14.3	23.7	22.3	20.9	10.5	22.1
9	Cardiolipin		2.6	1.3	1.0	3.9	3.4
10	Cerebrosides		1.4	2.0	2.5	4.3	1.1
12	Unknown other	1.0	0.7	3.0	2.2	12.2	2.8
	Total phospholipids	100.0	100.0	100.0	100.0	100.0	100.0

[a] Containing phosphorus and fatty acids.

Table 3b. Distribution of radioactivity among phospholipids of lymphocytes and thymocytes. Values expressed as percentages of radioactivity of total phospholipids.

TLC fraction	Phospholipid	Lymphocytes 3/L	48/L/1	54/L/3	54/L/6	Thymocytes 33/T	52/T
1	Start	5.1	0.1	0.7	0.9	5.3	0.8
2	Phosphatidic acid	5.1	1.0	1.1	1.0		0.9
3	Phosphatidyl serine	2.3	2.8	1.9	1.3	6.8	1.1
4	Phosphatidyl inositol	8.1	5.6	5.8	5.0	7.9	8.6
5–6	Sphingomyelin	15.5	8.1	10.6	12.3	6.0	2.2
7	Lecithin	48.8	55.3	47.7	49.7	39.8	58.4
8	Phosphatidyl ethanolamine	13.1	13.3	14.7	12.8	12.9	19.2
9	Cardiolipin			1.7	1.5	2.2	2.3
10	Cerebrosides		9.9	7.2	9.2	6.1	2.1
12	Unknown other	2.0	3.9	8.6	6.3	13.0	4.4
	Total phospholipids	100.0	100.0	100.0	100.0	100.0	100.0

Table 4. Distribution of mass and radioactivity among fatty acids in neutral lipids of human lymphocytes (3/L, 23/L, 48/L/3) and human thymocytes (33/T, 34/T, 36/T) incubated at +37°C with acetate-1-^{14}C. Glycerides and free fatty acids were isolated by silicic acid chromatography. After hydrolysis the fatty acids obtained were methylated and subjected to radio gas chromatography. Values are expressed as relative percentages of fatty acid methyl esters and of their radioactivity. The study does not include fatty acids with longer retention time than 24:2. Tr= Traces.

GLC peaks No.	Fatty acids [a]	3/L		23/L		48/L/3		33/T		34/T		36/T	
		Mass %	Act. %	Mass %	Act. %	Mass %	Act. %	Mass %	Act. %	Mass %	Act. %	Mass %	Act. %
1	12:0	0.8		2.3		1.9		1.7		1.3		1.2	
2	12:1					1.7							
3	14:0	1.9		2.9		2.5	1.4	3.6	4.4	3.9	2.1	2.3	3.2
4	14:1					2.6							
5	15:0			1.8		3.6							
6	16:0	25.0	2.9	24.8	5.3	14.4	4.5	22.1	10.9	24.5	6.7	19.1	8.7
7	16:1	1.0		4.3		7.2	1.3	4.4	2.1	4.3	0.8	2.5	1.5
8	18:0	25.1	34.8	16.5	20.3	10.2	9.5	9.0	3.6	9.4	7.6	30.3	8.5
9	18:1	17.5	17.1	11.6	23.8	17.0	12.3	39.8		40.4	3.5	25.4	6.5
10	18:2	8.9	3.1	3.1		6.5	1.4	14.3		8.8		4.5	3.2
11	20:0	Tr		1.4		1.7	1.2					1.4	
12	20:1	0.9	12.8	0.4	7.5	0.5	8.6	2.7		2.0	5.4	1.5	7.8
13	20:2		6.0	Tr	16.1	3.5	7.0				6.0	Tr	Tr
14	22:0 and 20:3 [b]	1.0	3.3	3.4	8.7	2.4	4.7	Tr	23.2		17.0	0.7	19.8
15	20:4, 22:0 and 22:1	13.7	5.5	10.4		8.6	4.5	2.4	6.0	2.5	7.5	1.7	Tr
16	22:2 and 23:0 [c]	4.2		13.7		12.1				2.1		5.6	
17	22:4 and 24:0			3.4	4.9	1.4				0.5		1.8	
18	22:4 and 24:1 [b]		14.5	Tr	13.4	1.1	31.5		49.8	0.3	43.4		40.8
19	22:5 and 24:2 [e]					1.1	12.1						
		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Denoted by chain length: number of double bonds.
[b] The radioactivity mainly confined to 20:3 (see also Ref. 19).
[c] It cannot be excluded that part of the peak was derived from ghost compounds, because experience in this laboratory with GLC-mass spectrometry indicates that in this region we sometimes have peaks as contaminants.
[d] The radioactivity mainly confined to 22:4 (see Ref. 19).
[e] The radioactivity confined to 22:5 (see Ref. 19).

most pronounced deviation in the incorporation of label into lipids, the relative incorporation into cholesterol being increased with a concomitant relative decrease in phospholipid radioactivity. This lymph portion (46/L/1) was obtained on the 10th day of drainage and it is known [5,17,18] that the proportion of larger lymphocytes in lymph increases with prolonged drainage.

Incorporation of acetate-1-^{14}C into phospholipids. The phospholipid composition (Table 3a) of lymphocytes and thymocytes varied among the experiments, but lecithin was consistently the largest fraction. The thymocytes contained a lower percentage of sphingomyelin than the lymphocytes.

Table 3b shows that the biosynthetic activity in the different experiments varied, but that most radioactivity was consistently incorporated into lecithin. In the experiments performed on thymocytes, a lower percentage of label was incorporated into sphingomyelin.

Incorporation of acetate-1-^{14}C into fatty acids of neutral lipids. Considerable quantitative differences have been obtained in the fatty acid composition of glycerides (Fraction 2 from the silicic acid column) among the experiments (Table 4). The main fatty acids were 16:0, 18:0, 18:1, and 18:2. In comparison with the lymphocytes the glycerides of the thymocytes contained more oleic acid and less erucic and/or arachidonic acid, but otherwise the fatty acid composition of glycerides from lymphocytes and thymocytes was similar.

The radioactivity distribution pattern among fatty acids of glycerides also exhibited considerable quantitative differences among the experiments. In thymocytes there were less radioactivity incorporated into stearic and oleic acids and more into the peaks corresponding to 20:3 + 22:0 and 22:4.

Incorporation of acetate-1-^{14}C into fatty acids of phospholipids. Large percentages of mass but comparatively small percentages of radioactivity were found in 16:0 in all experiments (Table 5).

Considerable quantities of mass and radioactivity were confined to 18:0 and 18:1. In 20:1 and 20:2 small percentages of mass and comparatively large percentages of radioactivity were found. In all cases a large percentage of radioactivity was found in peak 18, in which 24:1 and 22:4 were identified by mass spectrometry.

Qualitatively the fatty acid composition of phospholipids from lymphocytes and thymocytes showed great similarity. Quantitatively, the mass distribution among the fatty acids differed in all the experiments, but the differences were not specially pronounced between thymocytes and lymphocytes. Quantitative differences in the distribution of radioactivity among the fatty acids was found among all the cases. A difference between thymocytes and lymphocytes was found for 20:3 and/or 22:0.

Incorporation of acetate-1-^{14}C into fatty acids of individual phospholipids. The distribution of mass and radioactivity among the fatty acids of the individual phospholipids was quite different (Figs. 2a and 2b).

The fatty acid composition of lecithin was similar in lymphocytes and thymocytes (Table 6). The radioactivity distribution shows that in lymphocytes most radioactivity was found in 18:0 and in 18:1, and in 48/L/3 also fatty acids with 20 carbon atoms, while a low percentage of radioactivity was found in 16:0. In the thymocytes almost equal amounts of radioactivity were found in 16:0 and 18:0.

Table 5. Distribution of mass and radioactivity among the fatty acids in phospholipids of lymphocytes (45/L, 46/L/2, 48/L/3) and thymocytes (36/T) incubated at +37°C with acetate-1-^{14}C. The phospholipids isolated by silicic acid chromatography were hydrolyzed and the fatty acids obtained were methylated and subjected to radio gas chromatography. Values are expressed as relative percentages of fatty acid methylesters and their radioactivity. The study does not include fatty acids with longer retention times than 24:2. Tr=Traces.

GLC peaks No.	Fatty acids	45/L		46/L/2		48/L/3		36/T	
		Mass %	Act. %	Mass %	Act. %	Mass %	Act. %	Mass %	Act. %
1	12:0	1.3		0.3		0.2		0.2	0.6
2	12:1	1.6				0.2		Tr	
3	14:0	0.4		0.8		0.3		0.5	2.2
4	14:1	2.1				0.3		Tr	
5	15:0	2.7		0.7		2.9		2.5	1.2
6	16:0	26.2	5.6	28.7	11.3	21.7	4.9	16.2	8.5
7	16:1	4.3		1.3		1.2	1.6	1.1	
8	18:0	22.8	39.9	20.3	37.5	19.1	21.9	21.5	21.8
9	18:1	15.8	18.3	24.4	27.3	16.9	18.7	21.3	11.7
10	18:2	6.8	3.5	5.9	3.4	10.5	3.3	6.9	3.4
11	20:0	1.1		Tr		Tr		Tr	
12	20:1	Tr	9.6	1.0	8.3	0.7	7.5	1.9	4.9
13	20:2	1.6	9.8	Tr		1.1	16.6	0.2	3.0
14	20:3 and 22:0 [a]	1.5	2.6	2.0		2.3	5.1	4.4	16.1
15	20:4, 22:0 and 22:1 [b]	9.2	Tr	9.4		17.8	3.5	20.1	8.8
16	23:0 and 22:2	Tr		Tr		0.5		1.8	
17	22:4 and 24:0	0.1		0.6		0.6			
18	22:4 and 24:1 [c]	1.6	10.7	3.4	12.2	1.6	10.8	1.4	17.8
19	22:5 and 24:2 [d]	0.9	Tr	1.2		2.1	6.1	Tr	
		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Mass and radioactivity mainly confined to 20:3 (see also Ref. 19).
[b] Mass mainly confined to 20:4 (see also Ref. 19).
[c] Radioactivity mainly confined to 24:1 (see also Ref. 19).
[d] Radioactivity confined to 24:2 (see also Ref. 19).

Table 6. Mass and radioactivity distribution among the fatty acids of lecithin from lymphocytes (3/L, 48/L/3) and thymocytes (36/T) incubated with acetate-1-^{14}C. Values are expressed as percentages of total mass and radioactivity of fatty acids in lecithin.

	Lymphocytes				Thymocytes	
	3/L		48/L/3		36/T	
	Mass %	Radio-activity %	Mass %	Radio-activity %	Mass %	Radio-activity %
14:0	0.3		0.8			
15:0	0.4		0.4			
16:0	41.0	5.6	51.9	11.1	51.4	46.3
16:1	Tr		3.9		1.4	
18:0	19.8	52.1	15.9	31.2	16.9	53.7
18:1	16.9	29.4	21.0	24.1	17.0	
18:2	7.6	5.0	4.1	3.9	3.8	
20:0						
20:1	1.3	7.9	2.0	15.2	1.3	
20:2	0.6			11.7		
20:3	1.2			2.8	2.2	
20:4	10.9				6.0	
	100.0	100.0	100.0	100.0	100.0	100.0

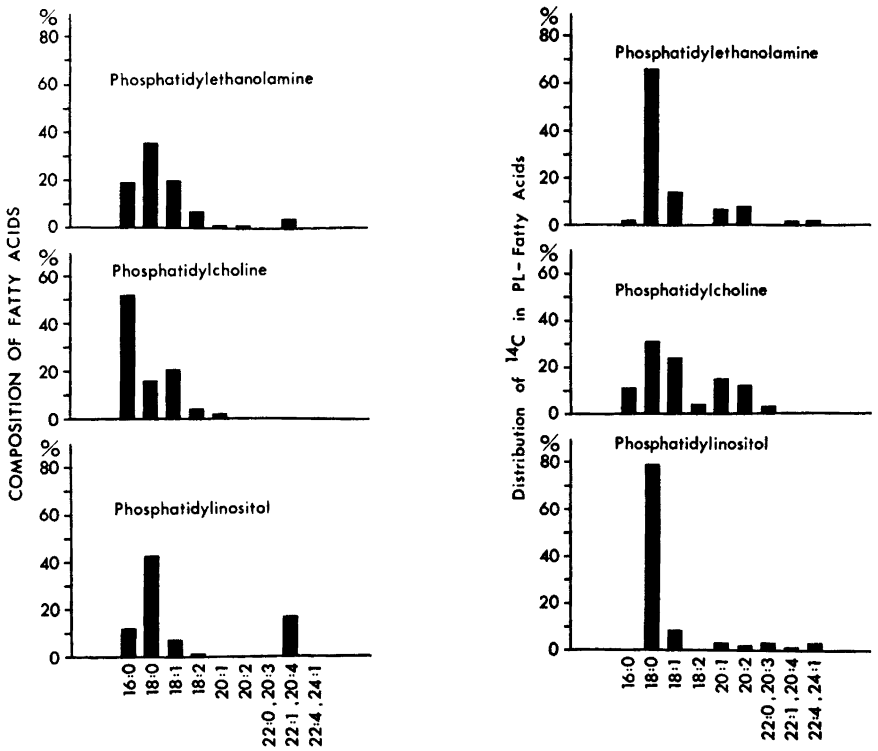

Fig. 2a–b. Composition of the main fatty acids and distribution of ^{14}C, in the main fatty acids of phosphatidyl ethanolamine, phosphatidyl chloline, and phosphatidyl inositol from human lymphocytes incubated with acetate-1-^{14}C.

DISCUSSION

This study indicates that lipid composition and biosynthesis is similar for lymphocytes and thymocytes. Among the different experiments with lymphocytes and thymocytes there were, however, some quantitative differences both in lipid composition and lipid synthesis.

Except for experiment 33/T, the phospholipids constituted the main part of the total lipids. This finding is in accordance with earlier results obtained for human lymphocytes,[1] leucocytes,[20] and erythrocytes.[21] Gottfried,[22] however, found lower values for the phospholipid content in his studies on human lymphocytes and leucocytes.

In 5 out of 7 experiments with lymphocytes the main part of label was incorporated into the phospholipids. In one experiment (46/L/1) where the lymphocytes were obtained late during the drainage only 30 % of the label of total lipids was recovered from the phospholipids. In the experiments with thymocytes, 49 and 36 % of the total radioactivity were recovered in the phospholipids.

Malamos *et al.*[23] and Kidson[24] found that in normal leucocytes more acetate-1-^{14}C was incorporated into neutral lipids than into phospholipids, while in leucemic leucocytes the main part of radioactivity was recovered from the phospholipids.

The diverging results may be explained by differences in cell clones and cell condition, but type and composition of media may also influence the results.[25,26]

The composition of phospholipids in lymphocytes and thymocytes was similar and mainly in agreement with values obtained by Gottfried[22] and Huber *et al.*[27] Sphingomyelin, which is most extensively found in plasma membranes,[28] seemed to make up a larger part of the phospholipids in the lymphocytes than in the thymocytes. Phosphatidyl ethanolamine, on account of the high content of polyunsaturated acids, is one of the most easily oxidized lipids[29] and in spite of precautions it cannot be excluded that the low content of this lipid in experiment 3/L and 33/T may be due to auto-oxidation.

Proportionately less radioactivity was incorporated into sphingomyelin in the thymocytes than in the lymphocytes, but otherwise the radioactivity distribution among the phospholipids was similar and in accordance with the results of Huber *et al.*[27]

The variability in the mass and radioactivity pattern of the fatty acids obtained for the phospholipids and the glycerides both among the lymphocyte and thymocyte experiments may reflect different proportions of subfractions of the lipids analyzed but may also be due to the different living conditions of cells before and during incubation.[26]

The mass and radioactivity distribution among the fatty acids of glycerides and phospholipids was mainly similar for thymocytes and lymphocytes and in accordance with earlier results obtained for lymphocytes.[1] In thymocytes, however, a larger amount of radioactivity was confined to 20:3 of glycerides and phospholipids and to 16:0 of lecithin.

The radioactivity distribution among fatty acids indicates that the label is incorporated into fatty acids mainly by chain elongation. This assumption is in accordance with earlier studies on lymphocytes[1] and leucocytes.[30,31] Majerus *et al.*[31] reported that the human leucocytes were unable to synthetize fatty acids *de novo*. The leucocytes lacked acetyl CoA carboxylase which, however, was detected in leucemic blast cells. The high percentage of radioactivity incorporated into the palmitic acid of thymocyte lecithin may suggest that a fatty acid synthesis *de novo* also is possible. Because of the small amounts of material available it was not possible to further elucidate this problem.

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