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Sterol and Phospholipid Biosynthesis in Phytohemagglutinin Stimulated Human Lymphocytes

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The incorporation of acetate-1-^{14}C into sterols and phospholipids of PHA-stimulated * human lymphocytes was studied *in vitro* in lymph samples from a thoracic duct cannulated patient.

The PHA-stimulated lymphocytes incorporated more radioactivity into sterols and phospholipids than the control lymphocytes.

The cholesterol was labelled in both groups of lymphocytes and to a significantly greater extent in the PHA-stimulated lymphocytes. However, the highest specific radioactivity was located in the lathosterol fraction of the PHA-stimulated cells.

Among the phospholipids the phosphatidyl inositol and sphingomyelin exhibited the most pronounced increase of radioactivity.

Many biological membranes contain an appreciable quantity of cholesterol in addition to phospholipids, proteins, *etc.* The interaction of cholesterol with phospholipids is of considerable biological importance and many studies have been made to gain an understanding of the function of cholesterol in biological membranes. As early as in 1908 [1] it was suggested that the cholesterol present in animal tissues was in some way associated with the phospholipids in a liquid crystalline structure. For recent reviews of the interaction of cholesterol and phospholipids in biological systems, see Ref. 2. In recent work on phospholipids and steroids, Van Deenen [3] attempted to correlate phospholipid monolayer characteristics with specific biomembrane function.

The composition of the lipid core in a given membrane is biogenetically determined to a certain extent, but also a number of environmental factors are known to influence the lipid composition of normal interface. In our earlier studies, a pronounced influence on the phospholipid biosynthesis was observed in lymphocytes after treatment with phytohemagglutinin.[4] In the

* *List of abbreviations.* BSTFA, bis-(trimethylsilyl)-trifluoro-acetamide; DCDMS, dichlorodimethylsilane; dimethyl-POPOP, 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene; FFA, free fatty acids; PEGS, polyethyleneglycolsuccinate; PHA, phytohemagglutinin; PPO, 2,5-diphenyloxazole; QF-1, fluorosilicone; Se-30, methylsilicone; TMCS, trimethylchlorosilane; TMS, trimethylsilyl.

present investigation, we have searched for alterations both in sterols and phospholipid biosynthesis in the lymphocytes after treatment with PHA. It was found that striking variations can be induced in these membraneous lipids, indicating that PHA-stimulated lymphocytes might be used as a relevant model system for improving our insight into the relations between the structure and function of the lipids present in natural membranes. Sterol biosynthesis of PHA-stimulated and non-stimulated lymphocytes has been compared. In addition, an analysis of the biosynthesized sterols has been carried out with the aid of gas chromatography and mass spectrometry.

EXPERIMENTAL

Lymphocytes and incubation procedure. Cannulation of the thoracic duct was performed in a 36-year-old woman suffering from *myasthenia gravis* in order to deprive the patient of lymph. Details of the treatment of this patient with a therapeutic lymph cannulation are described elsewhere.[5] The first 1000 ml of lymph were collected under sterile conditions into bottles kept at +4°C, while the patient was fasting. 2 IU heparin (Vitrum, Sweden) per ml lymph were added. The lymph contained 10 000 lymphocytes, 100 granulocytes, and 7000 erythrocytes per μl.

After thorough mixing, the lymph was divided into two equal portions. The lymph portions were transferred to two sterile culture vessels (Fernbach, diam. 200 mm. Grave, Sweden). The depth of the lymph in each vessel was 18 mm. 25 000 IU benzylpenicillin (Kabi, Sweden) were added to each vessel and 0.01 ml phytohemagglutinin (Wellcome Foundation, England) per ml lymph was added to one of the lymph portions. The culture vessels were closed with rubber stoppers equipped with needles for gas exchange. The lymph portions were then incubated for 18 h at +37°C in a shaking water bath. During the incubation the lymph portions were flushed with 5 % CO_2 in O_2. After 12 h, 2 μC acetate-1-^{14}C (specific activity 61 mCi/mM, The Radiochemical Centre, Amersham, England) per ml lymph were added to both lymph portions, and the incubations continued as before for 6 h. The cells were isolated by mild centrifugation at about 150 *g* for 10 min. The erythrocytes were hemolyzed by exposure to 0.3 % saline for 20 sec. The lymphocytes were then washed three times with 0.9 % saline. The lymphocytes were isolated from the saline by centrifugation at 150 *g* for 5 – 10 min. All centrifugations were performed at +4°C.

Extraction and silicic acid separation. The separated and washed lymphocytes were resuspended in saline and disrupted by freezing and thawing. The lipids were extracted according to Folch *et al.*[6] Aliquots of the total lipids were weighed and measured for radioactivity. Silicic acid (SiO_2) separations were carried out on HCl, water, chloroform and methanol washed Silicar 200 – 325 mesh (Mallinckrodt). Three fractions were eluted: (1) pentane:benzene 85:15 containing hydrocarbons and cholesterol esters; (2) chloroform containing glycerides, free cholesterol, and free fatty acids; and (3) methanol which elutes phospholipids and other polar lipids. Aliquots of these separated lipids were also weighed and measured for ^{14}C.

Thin layer chromatography. The plates were prepared with an automatic TLC-coater (Camag, Muttenz, Switzerland). Air-dried Silica Gel G (Merck, Darmstadt, Germany) plates (0.25 mm layer thickness) were heated for 2 h at 110° before use. The cholesterol ester and free cholesterol fractions from the silicic acid separation were chromatographed with pentane:ether 9:1 (system 1). The phospholipids were chromatographed with chloroform:methanol:13 N ammonia 14:6:1. The detection reagent was $(NH_4)_2SO_4$ 100 g and H_2SO_4 5 ml made up to a volume of 500 ml with water. The plates were charred 90 min at 180°C. Transmission densitometry was performed on a Vitraton TLD 100 densitometer (Vitatron, Dieren, Holland). Conditions and quantitative evaluations will be described elsewhere.[7] Alumina – $AgNO_3$ plates were prepared by mixing 35 g Alumina Woelm neutral (M. Woelm-Eschwege, Germany) with a solution of 10.5 g $AgNO_3$ in 30 ml water and spreading it on three 20 × 20 cm glass plates to a thickness of 0.25 mm. The plates were air-dried in darkness and activated at 120°C for 30 min just prior to

sample application. The developing phase was chloroform:acetone 65:35 (system 2). The spots were visualized by charring. The R_F values relative to cholestane were 0.44 for cholesterol and 0.69 for lathosterol. Squalene and 7-dehydrocholesterol remained at the start.

Water was used as a detection reagent on the TLC plates (white spots on a semi-translucent background) and the lipids were isolated by scraping off the marked areas with a razor blade into centrifuge tubes and extracted after shaking vigorously with 5 ml developing phase. The tubes were centrifuged and the procedure repeated 5 times. The evaporated combined extracts were reextracted with hexane or chloroform and filtered through HCl and methanol washed mineral fibres (1 μ, Bilsom International AB, Billesholm, Sweden).

Assay of radioactivity. Detection and assay of the radioactivity on the thin layer plates were carried out with a Berthold Thin-Layer Scanner II from Berthold Laboratorium, Wildbad, Germany. The weights of lipid fractions and sterols were determined by weighing aliquots of solutions with a Cahn Microbalance or a Mettler Ultra Microbalance UM 7 (Greifensee-Zürich, Switzerland). A Packard Tri-Carb liquid scintillation spectrometer model 3003 with an external standard was used for the assay of the radioactivity of aliquots of the lipid solutions. The scintillator mixture used consisted of 3.5 g PPO, 0.210 g POPOP, and 700 ml toluene.

Radio gas chromatography. The separation of labelled sterols was carried out on an F & M Biomedical Model 400 gas chromatograph equipped with an RGC 170 hydrocracking continuous-flow reaction tube with a proportional counter tube and a radiation measurement set-up (LB 2411) from Berthold Laboratorium, Wildbad, Germany. We have described a modified apparatus and the technique used elsewhere.[8] The U-shaped glass columns were 2 m$\times$3 mm, washed with conc. HCl and siliconized with 5 % DCDMS in toluene for 2 h. Acid-washed Chromosorb W, 80–100 mesh, was treated with 5 % DCDMS and 3 % TMCS in toluene for 12 h, washed with methanol and heated at 150°C for 12 h. The stationary phases used were QF-1 (4 % w/w) at 220°C, Se 30 (3 % w/w) at 250°C, and PEGS (14 % w/w) at 200°C. The gas flow conditions were the same as for fatty acid methylesters as described elsewhere.[8] The retention times relative to cholestane of some sterols are listed in Table 6. The TMS derivatives were prepared with BSTFA as described earlier.[9]

GLC-mass spectrometry. A QF-1 and an Se 30 column were prepared in the same way as described for radio gas chromatography, checked for separation behavior and used in the combined GLC–MS instrument LKB 9000. Operating conditions: Column 220 and 250°C, molecule separator 250°, ion source 270° and electron energy 70 eV.

Chemicals. Cholestane, cholesterol, squalene, and cholestanol were obtained from Koch-Light Laboratories, Ltd., Colnbrook, England. Lathosterol from Calbiochem, California Corporation, and 7-dehydrocholesterol from Mann Research Laboratories, Inc., New York. BSTFA was obtained from Pierce Chem. Co., Rockford, Ill. Solvents were of analytical grade and distilled with Vigreux columns before use.

RESULTS

In Table 1 are shown the results obtained from silicic acid and thin layer chromatograms of the lipids extracted from lymphocytes incubated with or without PHA for 18 h and to which acetate-1-^{14}C was added during the last 6 h of incubation. The total incorporation of radioactivity into the lipids of the stimulated lymphocytes was about 5 times higher that in the control experiment. In the control experiment the main part of the activity was found in the phospholipid fraction. The stimulated lymphocytes showed a considerably higher incorporation of label into the sterols in comparison with the control experiment. This sterol fraction, which was eluted together with the glycerides and FFA from the silicic acid column, was rechromatographed on silica gel G plates, and the sterols were isolated from the thin layer, hydrolyzed and separated from acidic components.

Table 1. Mass and radioactivity distribution among lipid classes from PHA stimulated and non-stimulated lymphocytes.

	Control			PHA-stimulated		
	Mass %	Radioactivity dpm × 10^3	%	Mass %	Radioactivity dpm × 10^3	%
Hydrocarbons [a]	1.5	70	1.1	3.0	518	1.5
Cholesterol esters [b]	3.5	83	1.3	2.8	794	2.3
Monoglycerides [c]	3.6	665	10.4	4.6	3 487	10.1
Cholesterol [d]	7.8	1087	17.0	8.0	11 427	33.1
FFA	1.2	364	5.7	1.1	1 519	4.4
Triglycerides	5.4	748	11.7	11.4	6 387	18.5
Phospholipids	77.0	3375	52.8	69.1	10 391	30.1
Total lipids	100.0	6392	100.0	100.0	34 523	100.0

[a] Front fraction from TLC, containing squalene and other non-polar compounds. [b] Also containing other sterol esters. [c] Not completely separated and identified. [d] Also containing other sterols.

The pentane-benzene fraction from the silicic acid column was separated into sterol esters and hydrocarbons on a silica gel G plate using solvent system 1. The sterol esters and the hydrocarbon fraction of the PHA-stimulated lymphocytes exhibited an increased labelling compared to the non-stimulated controls (Table 1 and Fig. 4). Fig. 4C also shows a sterol ester fraction from lymphocytes stimulated with PHA for 42 h and then incubated with PHA and acetate-1-^{14}C for 6 h. The incorporation of label into the hydrocarbon fraction had risen, whereas the radioactivity of the sterol esters had nearly disappeared.

The mass of the total sterols was esterified to about 20 % in the control lymphocytes. Similar results have been obtained by d'Hollander and Chevallier [10] studying sterol biosynthesis and esterification of different rat

Table 2. Total number of dpm × 10^3 incorporated by human thoracic duct lymphocytes into sterols after incubation with acetate-1-^{14}C.

	Control	PHA-stimulated
Free sterols	1087	11 427
Sterol esters	21 [a]	413 [a]
Free sterols		
Cholesterol	559	7 153
Lathosterol	528	2 925
Esterified sterols		
Cholesterol	5	263
Lathosterol	13	109

[a] Expressed as the activity in the sterol moiety.

tissues. The stimulated lymphocytes exhibited a similar esterification (16 %). The mass of the total lipids increased about 25 % by stimulation. The triglycerides were mainly responsible for this increase. The mass of the free sterols, however, also increased by about 25 % compared with the non-stimulated lymphocytes. The total incorporation of activity into the free sterol pool increased 10 times by PHA-stimulation and was distributed between cholesterol and lathosterol in a ratio of 2:1 (Table 2). After subtraction of the activity found in the fatty acid moiety, the sterol esters exhibited an increase of incorporation by 20 times. This increase was more pronounced for cholesterol (50 times) than for lathosterol (9 times).

None-sterified sterols. The radioactive free sterols from stimulated and non-stimulated lymphocytes isolated from thin layer plates (solvent system 1) were purified by alkaline hydrolysis, silylated with BSTFA and rechromatographed with the same TLC system. All activity moved according to the R_F of the TMS derivative of cholesterol. Radio gas chromatographic analysis with a QF-1 column demonstrated that the main part of the radioactive TMS derivatives exhibited the retention time of cholesterol TMS ether. A minor part of the activity appeared as a tail of the cholesterol peak. No other radioactive peaks were obtained.

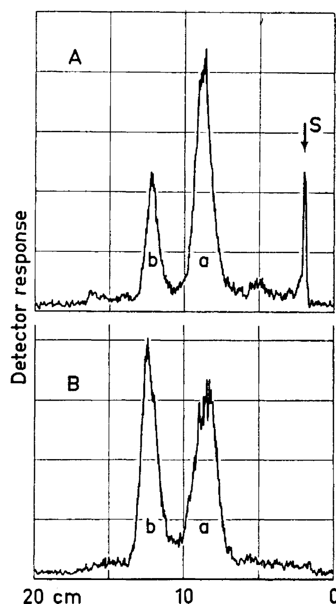

Fig. 1. Thin layer radio scan of the free sterols separated on silver nitrate coated plates. A, PHA-stimulated, and B, non-stimulated lymphocytes. Cholesterol, a; and lathosterol, b. S indicates the start point. The plates were developed in chloroform:acetone 65:35.

Fig. 1 shows radioactivity scans of the free sterols separated on an alumina-$AgNO_3$ thin-layer plate: A, from stimulated lymphocytes, and B, from the control lymphocytes. Peak a has the retention factor of cholesterol. The two radioactive compounds were isolated from the thin layer, and peak

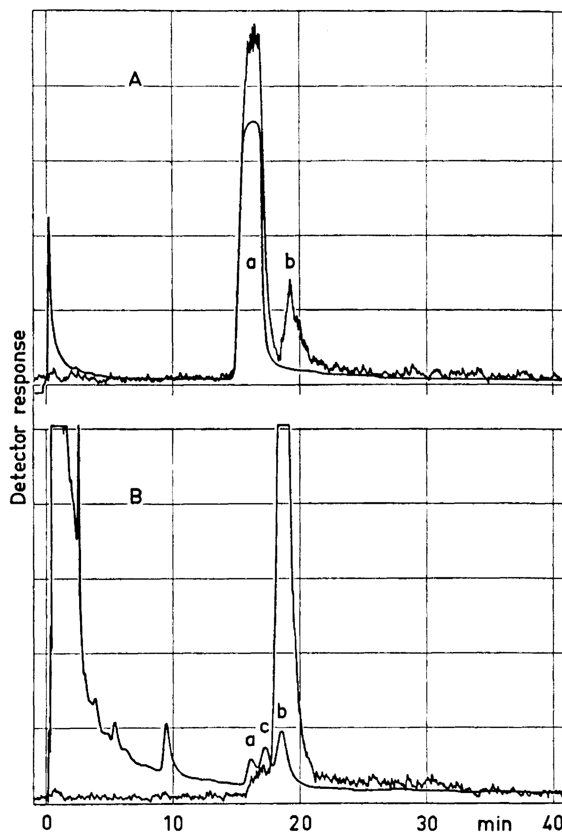

Fig. 2. Radio gas chromatograms of TMS derivatives of the free sterols from stimulated lymphocytes which were isolated from $AgNO_3$ TLC plates. A corresponds to peak a, and B to peak b in Fig. 1A. Peak a has been identified as cholesterol, b as lathosterol, and c as cholestanol. A QF-1 column was used. Conditions, see text.

a (Fig. 1A) had about 99 % of the mass with a specific activity of 3027×10^3 dpm/mg (Table 4). The small amount of compound b (Fig. 1A) and the presence of contaminants did not permit accurate weight determination. Fig. 2A shows a radio gas chromatogram of the TMS derivative of compound a (Fig. 1A). A QF-1 column, which separates cholesterol from cholestanol, was used. The main peak had the retention time of cholesterol TMS ether. The radioactive peak b originates from an incomplete separation on the alumina-$AgNO_3$ plate. Compound a (Fig. 1A) was subjected to GLC-mass spectrometric examination using a QF-1 column with chromatographic behavior identical to the column on the radio gas chromatograph. Cholesterol TMS ether could be identified. No other ions which would refer to additional TMS derivatives were found in the mass spectra. Finally, Fig. 2B shows the radio gas chromatogram of compound b TMS derivatives (Fig. 1A). It is obvious from the figure that compound b, with the retention time of lathosterol TMS ether,

carries the label. The retention time of compound c coincides with that of cholestanol TMS ether. Compound a, cholesterol TMS ether, originated from the incomplete separation on the alumina-$AgNO_3$ plate. All three compounds were identified by GLC-mass spectrometry.

The material remaining at the origin on the TLC plate shown in Fig. 1A was analyzed by TLC and radio gas chromatography. The results suggested that the fraction contained a labelled sterol with hydroxyl function. The preliminary data suggested the presence of 7-dehydrocholesterol, a common precursor in the 24-dihydro series.

Esterified sterols. The sterol esters from the stimulated lymphocytes (Fig. 4A, a) were hydrolyzed with 5 % KOH in 95 % ethanol for 1 h on a boiling water bath under nitrogen. 52 % of the activity was found in the sterol moiety. The residual activity consisted of acidic components and was analyzed

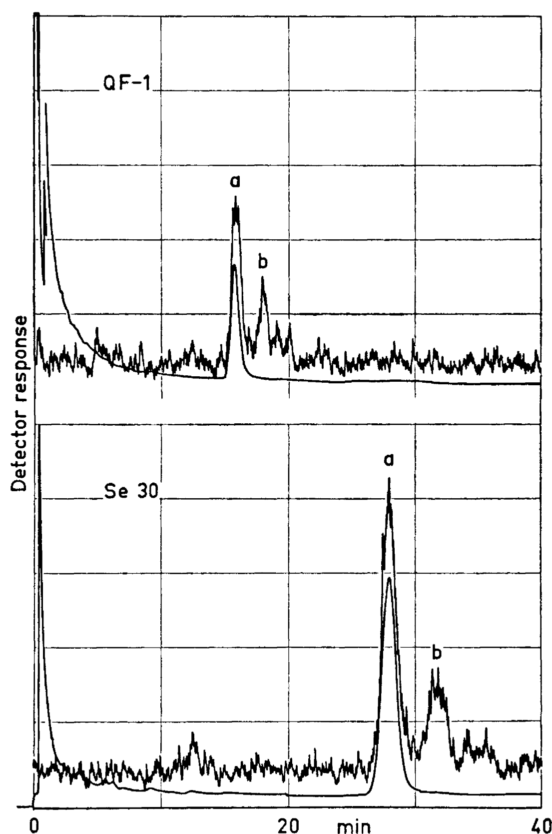

Fig. 3. Radio gas chromatograms of the esterified sterols on two different columns. The TMS derivatives were prepared after alkaline hydrolysis of the sterol esters from the stimulated lymphocytes. a, identified as cholesterol, and b, compound with chromatographic behavior of lathosterol. Column conditions, see text.

Table 3. Percentage distribution of radioactivity incorporated by human thoracic duct lymphocytes into cholesterol and lathosterol after incubation with acetate-1-^{14}C.

	Free sterols		Esterified sterols	
	Cholesterol	Lathosterol	Cholesterol	Lathosterol
Control	51.4 [a]	48.6 [a]	22.2 [a]	59.2 [a]
PHA-stimulated	62.6 [a]	25.6 [a]	63.6 [a]	26.5 [b]

[a] Value obtained by TLC radioscanning. [b] Value obtained by radio gas chromatography.

by radio gas chromatography in respect to fatty acids. These results will be published later.[4] After silylation, the sterols were subjected to radio gas chromatography. Fig. 3 shows chromatograms on two different columns. Compound a (cholesterol TMS ether), which is responsible for 63.6 % of the activity and 99 % of the mass (Table 3), was also identified by mass spectrometry. The extremely small amount of compound b did not allow mass spectrometric identification but the retention time on two different GLC columns coincided with that of lathosterol. Two other radioactive sterols can be seen on the chromatograms. These are responsible for about 10 % of the activity.

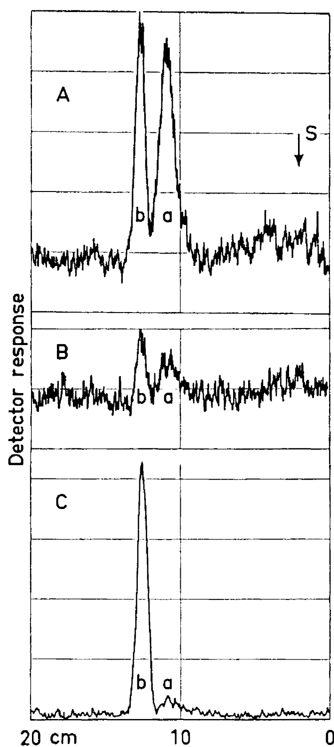

Fig 4. Thin layer radio scan of the cholesterol ester fraction obtained from SiO_2 chromatography. The silica gel G plates were developed with pentane:ether 9:1. A, stimulated lymphocytes; B, non-stimulated lymphocytes; C, lymphocytes incubated 48 h with PHA and with acetate-1-^{14}C added during the last 6 h of incubation. a, sterol esters, and b, hydrocarbons. The label of b has the chromatographic behavior of squalene.

No further attempt was made to identify these sterols, because additional material was not available. The sterol esters from the non-stimulated lymphocytes were also analyzed (Fig. 4B, a). The sterols obtained after alkaline hydrolysis contained 26 % of the radioactivity and were separated on an alumina-$AgNO_3$ plate. According to the radio scan (not shown) recorded from the plate, three radioactive compounds were present: a start fraction containing 18.6 % of the activity (not further analyzed), cholesterol with only 22.2 % of the activity (see Table 3) and lathosterol with 59.2 %. The lathosterol was submitted to radio gas chromatography as the TMS derivate on an Se 30 column. Only one ^{14}C-peak appeared with the retention time of lathosterol TMS ether. The cholesterol isolated from the alumina plate was identified by mass spectrometry. The specific activity was too low for radio gas chromatographic analysis. The identification of esterified lathosterol could only be performed with the aid of radio gas chromatography and radioactivity scanning of thin layer chromatograms.

Hydrocarbons. The hydrocarbon fraction (TLC front fraction) obtained from the silicic acid and TLC chromatograms (Fig. 4, A – C) was alkaline hydrolyzed and the non-saponifiable lipids, containing 96 – 100 % of the radioactivity, were analyzed in respect to the label. It was found that the mass associated with the radioactivity was very small and mass spectral identification could not be performed. The retention factor of the major activity agreed with that of squalene in TLC systems 1 and 2. The label was further analyzed on 3 different radio gas chromatographic columns with QF-1, Se 30, and polyethyleneglycol succinate as the liquid phase. A silylated sample was analyzed on QF-1 and Se 30 columns. The latter column would lengthen the retention time of TMS derivatives and the former shorten it in respect to the parent compound. About 10 % of the label was found to be non-identical with squalene according to the identification methods used, but rather consisted of at least 3 other compounds. No difference in composition of the label was found between the lipids of the stimulated and non-stimulated lymphocytes.

Phospholipids. In Table 5 the distribution of mass and radioactivity among the individual phospholipids from the PHA-stimulated and the non-stimulated lymphocytes is given. The method used for the determination of mass and activity of the individual phospholipids separated on thin layer

Table 4. Specific activity of cholesterol biosynthesized by human thoracic duct lymphocytes after incubation with acetate-1-^{14}C.

	Control dpm × 10^3/mg	PHA-stimulated dpm × 10^3/mg
Free cholesterol	119	3027
Esterified [a] cholesterol	13	471

[a] Expressed as the activity found in the sterol moiety after alkaline hydrolysis.

Table 5. Mass and radioactivity distribution among phospholipids from PHA stimulated and non-stimulated lymphocytes.

	Control		PHA-stimulated	
	Mass %	Radioactivity dpm × 10^3	Mass %	Radioactivity dpm × 10^3
Start fraction	5.9	118	7.1	468
Phosphatidic acid	5.8	145	5.9	436
Phosphatidyl serine	6.3	152	5.6	436
Phosphatidyl inositol	9.7	219	9.8	1 205
Sphingomyelin	17.4	314	15.4	1 309
Phosphatidyl choline	36.4	1 525	37.0	4 146
Phosphatidyl ethanolamine	15.0	537	14.7	1 746
Cardiolipin	0.4	44	0.5	94
Cerebrosides	3.1	321	4.0	551
Total phospholipids	100.0	3 375	100.0	10 391

plates will be discussed elsewhere.[7,11] The total incorporated radioactivity in the phospholipids of the stimulated lymphocytes was about 3 times higher than that of the control experiment (Table 5). A more pronounced increase of radioactivity was found for phosphatidyl inositol and sphingomyelin. After hydrolysis of the phospholipids isolated from the thin-layer plates, almost 100 % of the label was found in the fatty acid moiety.[4]

DISCUSSION

The present report deals with data obtained from studies on sterol and phospholipid biosynthesis in human lymphocytes. Because there are few data available on the composition and synthesis of the different lipid classes in human lymphocytes,[12] we have analyzed the sterols and phospholipids rather extensively. This approach can help to explain the interrelationships among different types of cellular lipids and also elucidate the metabolic functions of lipids.

In a recent investigation from this laboratory,[7] the lipid composition and synthesis in human lymphocytes and thymocytes has been compared. A great

Table 6. GLC retention times of some sterols relative to cholestane.

Compound	Se 30 250°C	QF-1 210°C	PEGS 200°C
Cholestane	1.00	1.00	1.00
Squalene	1.00	0.66	0.90
Cholesterol TMS ether	1.81	2.09	–
Cholestanol TMS ether	1.81	2.27	–
Δ^7-Cholesten-3β-ol TMS ether	2.01	2.74	–
$\Delta^{5,7}$-Cholestadien-3β-ol TMS ether	2.11		

similarity in the lipid composition and metabolism was found in these two types of lymphocytes, although minor differences were observed.

From Tables 1 and 5 can be seen that from a qualitative point of view, non-stimulated and PHA-stimulated lymphocytes have a very similar lipid composition.

This investigation further confirmed the results of our previous investigations that human lymphocytes have a very active lipid synthesis [7,13] and that lipid synthesis is stimulated by PHA.[14]

With regard to the sterol synthesis the data in Tables 2 and 3 indicate that more acetate-1-^{14}C is incorporated into the cholesterol of the PHA-stimulated cells than into other sterols. The specific activity of free lathosterol in the stimulated lymphocytes was, however, about 200 times higher than that of cholesterol as calculated from Fig. 2 (the sensitivity of mass detector was 20 times less in Fig. 2A than in Fig. 2B). The specific activity of lathosterol in the control experiment could not be estimated.

From Table 4 it is obvious that the specific activity of free cholesterol was considerably higher than that of ester cholesterol but in both sterol pools the incorporation of label was increased after PHA-stimulation.

Table 2 shows the total incorporation of label into sterols. It is obvious from the control experiment that in the free sterol pool about the same amount of radioactivity was incorporated into cholesterol and lathosterol, but in the esterified sterol pool lathosterol was labelled 2.5 times as much as cholesterol. In the stimulated lymphocytes, more radioactivity was incorporated into cholesterol than into lathosterol both according to the free and esterified sterol pools. The results suggest a proportionally larger synthesis and esterification of cholesterol in PHA-stimulated lymphocytes.

It is known that PHA stimulates the growth and cell division of lymphocytes.[15] The preferential labelling of the free cholesterol in comparison with the ester cholesterol may be due to a compartmentalization of the lymphocytic sterols.[16] It is also possible that the synthesized sterols are preferentially used for membrane formation as it is generally assumed that cholesterol serves as a structural unit in cellular membranes [2,17] and that it seems to undergo little or no change in tissue culture cells.[18] The incorporation into membranes of lathosterol, 7-dehydrocholesterol, Δ^4-cholesten-3-one, and coprostanol has been reported by Rothblat and Buchko.[19]

The accelerated sterol synthesis of PHA-stimulated lymphocytes also supports the hypothesis of Chevallier and Lutton [20] that only during the formation of cells is there an active cholesterol synthesis.

Besides cholesterol and proteins, phospholipids are the main constituents of the cell membranes. From Table 5 it is obvious that PHA induced an increased phospholipid synthesis. Phosphatidyl inositol and sphingomyelin exhibited the most pronounced increase of radioactivity. The increased synthesis of the latter is of special interest because sphinomyelin seems to be preferentially associated with the plasma membrane.[21] A more detailed description of the influence of PHA on the lymphocyte phospholipids will be given elsewhere.[4]

The present results and those obtained earlier [4] clearly indicate that sterol and phospholipid biosynthesis from acetate-1-^{14}C is stimulated in the

presence of PHA. The observations on the increased incorporation of label into squalene and lathosterol after PHA-stimulation may indicate that PHA influences different steps in the synthesis of cholesterol. The exact nature of the mechanism involved in the PHA-stimulation of lymphocytes is unknown at present. It is possible, however, that the use of PHA as a stimulant of biosynthetic processes in lipid metabolism could be valuable as a tool for the elucidation of the sequences involved in membrane synthesis of lymphocytes.

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