Observations on the Acceleration of Fatty Acid Synthesis in Phytohemagglutinin-treated Lymphocytes

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Human thoracic duct lymphocytes have a very active lipid metabolism with a specificity for formation of higher fatty acids.[1] Exposure of small lymphocytes to phytohemagglutinin (PHA) leads to a stimulation of DNA, RNA, protein, carbohydrate and phospholipid synthesis.[2-5] The altered behaviour of the small lymphocyte after PHA-stimulation suggests a changed plasma membrane function [6-8] that may be associated with the phospholipid metabolism. There are still wide gaps in our basic knowledge of the distribution and metabolism of lipids in the lymphocytes. For these reasons a systematic study has been undertaken using human thoracic duct lymphocytes as model cells. Initial studies on the effect of phytohemagglutinin on the fatty acid synthesis are reported here.

Experimental. Cannulation of the thoracic duct was performed in connection with a scalene lymph-node biopsy on a patient with gastric carcinoma in order to examine lymph for cancer cells.[9]

The lymph was collected directly into a bottle kept at +4°C. 2 I.U. heparin (Vitrum, Sweden) per ml lymph was added. 300 ml of fresh lymph was collected for our investigations. The lymph contained 3200 lymphocytes, 50 granulocytes, and 400 erythrocytes per mm^3.

The lymph was divided into two equal portions, to one of which 0.01 ml phytohemagglutinin (Wellcome Foundation, England) per ml lymph was added. The lymph portions were incubated at +37°C in a shaking water bath. The lymph was continuously flushed with 5 % CO_2 in oxygen. After 12 h, 2 μCi acetate-1-^{14}C (specific activity 61 mCi/mmol, Radiochemical Centre, Amersham, England) per ml lymph was added to both portions, and the incubation continued as before for 6 h. The lymphocytes were then isolated by centrifugation and washed in saline three times. The lymphocytes were freezed and thawed. The total lipids were prepared by extraction with 19 volumes of chloroform-methanol 2:1 and washed according to Folch *et al.*[10] The fatty acids of the total lipids were obtained by hydrolysis and, after removal of the nonsaponifiable fraction, converted to their methylesters with diazomethane.

The radioactivity of the fatty acid methylesters was assayed by liquid scintillation on a Packard Tri-Carb Spectrometer and weights were determined on a micro-Cahn balance.

Simultaneous determinations of mass and radioactivity of the fatty acid methyl esters were performed by radio gas chromatography as recently described.[11] In this radio gas chromatographic procedure, the total effluent of a temperature-programmed gas chromatogram is passed through a hydro-cracking tube, followed by division of the produced methane by a splitter at room temperature. The splitter feeds an external flame ionization detector and a proportional detector. The system is also equipped with a special injection device which allows introduction of precise amounts of mass and radioactivity. The procedure allows the determination of specific activities of individual fatty acids in complex mixtures.

The different fatty acid methylesters were identified by their retention time, compared to that of reference fatty acid methylesters (Supelco Inc., Pa. USA). Masspectrometric identification is also in progress.

The chromatograms were calculated by cutting peaks from the recorder chart and weighing the paper for radioactivity. The areas of mass were measured by triangulation.

Results and comments. The specific radioactivity and the distribution of mass and radioactivity of fatty acids of non-stimulated and PHA-stimulated human thoracic duct lymphocytes is given in Table 1.

There is a very similar pattern of the fatty acid composition of the total lipids in nonstimulated and PHA-stimulated lymphocytes. The distribution of radioactivity, however, is changed. In the PHA-stimulated lymphocytes there appears a higher percentage of radioactivity in palmitic acid.

A comparison of the specific radioactivity in the fatty acids of non-stimulated and PHA-stimulated lymphocytes shows a significant rise of the specific radioactivity in all fatty acids of the PHA-stimulated lymphocytes, but the increase is most pronounced for palmitic acid. Recently performed experiments in this laboratory

Table 1. Distribution of mass and radioactivity and specific activities among the fatty acids of the total lipids of nonstimulated and PHA-stimulated human thoracic duct lymphocytes. 300 ml lymph containing 3200 lymphocytes per mm^3 was divided into two equal portions, to one of which 0.01 ml PHA/ml lymph was added. Both lymph portions were incubated for 18 h. After 12 h 2 μCi acetate-1-^{14}C was added per ml lymph to both portions.

Fatty acid	Mass distribution %		Radioactivity distribution %		Specific activity dpm/μg	
	Non-stimulated	PHA-stimulated	Non-stimulated	PHA-stimulated	Non-stimulated	PHA-stimulated
12:0	2.9	1.5	–	–	–	–
14:0	4.9	2.8	–	0.6	–	219
16:0	24.4	22.4	3.2	9.5	28	434
16:1	3.0	3.1	–	1.2	–	396
18:0	11.5	13.0	23.1	16.4	430	1291
18:1	24.3	20.5	6.6	8.1	58	404
18:2	13.1	14.6	–	–		
18:3+20:1	1.8	1.9	7.6	7.6	904	4172
20:2	traces	0.8	12.9	12.4	[a]	15857
20:3+22:0	1.2	2.1	3.9	4.2	696	2046
20:4+22:1	9.1	10.1	5.5	3.4	129	345
24:0	1.3	2.1	–	traces		
24:1+22:4	1.4	2.0	19.9	19.6	3042	10025
24:2+22:5	traces	1.7	11.8	13.6	[a]	8184
Unidentified	1.1	1.4	5.5	3.4	1070	2485
TLFA	100.0	100.0	100.0	100.0	214	1023

[a] Tracer amounts of mass do not permit calculation of the specific activity.

have shown that the detected selective acceleration of the incorporation rate of acetate-1-^{14}C into palmitic acid, when lymphocytes are exposed to PHA, is valid for both neutral lipids and phospholipids and is significant also after a 6 h treatment with PHA.

It has earlier been shown that the fatty acids of lymphocytes [1] and leucocytes [12] are mainly synthetized by chain elongation. It has also been shown that leucocytes are inable to synthetize fatty acids *de novo* because they lack acetyl CoA carboxylase.[13] This enzyme, however, was detected in leucemic blast cells.[13]

The described rise of specific activity of palmitic acid may be explained by an activation of *de novo* synthesis when lymphocytes are transformed to blast cells by PHA. Further experiments have to be done before this possibility can be verified.

The increased synthesis of palmitic acid may lead to changes in the structure of lipid constituents of the plasma membrane. The changed behaviour of the plasma membrane of lymphocytes exposed to PHA [6-8] may be elucidated by further investigations along the lines of this study.

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The Effect of Electron Scavengers on Photo-produced Electrons in Ethyleneglycol/Water Glass

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Several studies of electron scavenging in glassy media by means of optical absorption- and ESR-spectroscopy have been reported.[1-3] Trapped electrons may also be studied by thermoluminescence measurements since their recombination with positive ions upon heating may give rise to luminescence. It is therefore possible to carry out scavenging studies by means of thermoluminescence measurements. A problem that may be solved by this method is whether the recombining electrons have the same relative reactivity as the mobile electrons prior to trapping. In the present communication we report measurements of the effect of various electron scavengers on the yield of UV-induced trapped electrons as measured by optical absorption-spectroscopy and the effect of the same scavengers on the yield of UV-induced thermoluminescence.

Trapped electrons were produced by photoionization of tryptophan which was dissolved to a concentration of 5×10^{-4} mol/l in a mixture of ethyleneglycol and water (EG/H_2O) 1:1 by volume.[4] The samples were irradiated by 250 nm UV-light at 77 K and their optical densities and thermoluminescence glow curves observed upon heating were recorded by an apparatus described elsewhere.[5]

When an electron scavenger is present in the samples the electrons may be captured by it both on their way out from the positive ion prior to trapping and after being released from their traps. We will first look at the scavenging of the mobile electrons prior to trapping. Let r be the mean distance travelled by the electrons prior to trapping. We will suppose that a scavenger molecule which is within the distance r of a ionization reacts with the ejected electron with a probability k_m. If the scavenger molecules are distributed in the volumes $V = \frac{4}{3}\pi r^3$ according to Poisson's formula, this leads to the following equation for the yield G of trapped electrons:[10,11]

$$G_0/G = \exp(k_m V[S]) \quad (1)$$

where G_0 is the yield when no scavenger is present and [S] is the scavenger concentration. As may be seen from the stippled curves on Figs. 1 and 2 this equation seems to be in accordance with the experiments to a first approximation. However, in the case of CCl_3COOH and $ClCH_2COOH$ the experimental points for the highest scavenger concentrations lie distinctly below the theoretical curves. The reason for this is not understood. The relative values for k_m and the scavenger concentrations $[S]_{½}$ needed to halve the yield of trapped electrons can be found from the figures. The values are given in Table 1. The values of $[S]_{½}$ are about a factor 1.5 smaller than those reported by Steen *et al.*[3] in the case of X-ray induced ionization of EG/H_2O. This is in qualitative accordance with the findings of Hase and Kevan.[6] They attributed the difference in the values of $[S]_{½}$ for X-ray and UV-induced mobile electrons to spur formation in the case of X-rays. Thus the electrons produced in the spurs do not travel far enough to randomize before they become trapped.

The quenching of the thermoluminescence by scavengers include both the scavenging of mobile electrons prior to trapping and the scavenging of released, recombining electrons. The glow curve of tryptophan in EG/H_2O-glass irradiated at 77 K consists of two peaks.[4] Only a small part of the trapped electrons (less than 2 %) disappear in the temperature region