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Vitamin E deficiency and fatty acid turnover in erythrocyte membranes*

A criterion of experimental vitamin E (α-tocopherol) deficiency in rats is the susceptibility of erythrocytes to hemolysis by dialuric acid[1], an effect which can be reversed *in vitro* or *in vivo* by the addition of vitamin E to the blood.

The formation of a lipid peroxide has been implicated in this process[2], suggesting that α-tocopherol deficiency may affect lipid metabolism in the cell membrane. One plausible explanation for the apparently enhanced fragility of the erythrocyte membranes in a vitamin E deficiency is the possibility of a decrease in the free fatty acid pool as a result of lipid peroxidation, thus limiting the acylation of lysolecithin. The latter process is known to occur in normal erythrocytes[3–5] and could possibly represent an important mechanism by which the erythrocyte membrane is maintained.

As a test of this hypothesis we have examined in the present study the incorporation of [^{14}C]oleic acid into phospholipid (presumably through the acylation of a lysophosphatide by the free fatty acids) of isolated erythrocyte preparations from normal and vitamin E-deficient rats.

Female Wistar rats were raised on a vitamin E-free diet[6]. The control group had the same diet supplemented with 100 mg α-tocopherol per kg of food, a concentration which we have found to prevent, consistently, all symptoms of vitamin E deficiency. The experimental animals were used only after the level of hemolysis by dialuric acid[7] had reached 100% (usually after 8 weeks on diet). The corresponding control animals showed less than 10% hemolysis.

Erythrocytes from blood drawn from the aorta in the presence of added heparin, were collected by centrifugation at 800 × *g* for 10 min, washed several times with 0.154 M NaCl solution and resuspended at 25% (v/v) packed cells in 0.154 M NaCl. For some experiments erythrocyte ghosts were prepared as described[4] and also suspended at a 25% packing ratio.

Ghosts or intact cells were incubated with [1-^{14}C]oleic acid (10^5 counts/min per mμmole in 0.1 ml solution of bovine serum albumin, 3 mg/ml), for 1 h in a 3-ml reaction volume containing: ATP, 2 μmoles; CoA, 0.4 μmole; $MgCl_2$, 3 μmoles; and potassium phosphate buffer, 150 μmoles, pH 7.6, at 37°. The reaction was stopped by transferring the flasks to an ice bath and immediately adding 10 vol. of cold 10^{-4} M EDTA solution. The cells or ghosts were washed several times in 10^{-4} M EDTA–0.154 M NaCl solution, and resuspended to a final volume of 1 ml. Lipids were extracted and separated into phospholipids, neutral lipids, and free fatty acid fractions as described previously[4]. Radioactivity in each fraction was determined as published[4]. The results of preliminary experiments established that the rate of incorporation of oleic acid under the above conditions was linear with time.

The phospholipid fraction was further resolved by thin-layer chromatography on silica gel plates, developed in chloroform–methanol–acetic acid–water (65:28:8:4, by vol.)[8]. The resulting separated phospholipids (which were efficiently resolved as compared with authentic purified samples, kindly supplied by Dr. M. SILVER) were detected by exposure to iodine vapors, removed by scraping, and transferred to vials

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TABLE I

INCORPORATION OF [1-^{14}C]OLEIC ACID INTO PHOSPHOLIPIDS OF ERYTHROCYTE MEMBRANES

Incubation mixture as indicated in text. The ghosts and intact cells of each experiment were obtained from different animals (the blood of 2 animals was generally pooled for each experiment) and the experiments performed independently at different times.

Expt. No.	*Membrane source*	*Incubation time (h)*	*Counts/min per μg P*	
			Tocopherol-deficient	*Tocopherol-supplemented*
1	Ghosts	2	1681	1742
2	Ghosts	2	2349	1549
3	Intact cells	1	2015	1885
4	Intact cells	1	2115	1765
5	Intact cells	2	2310	2440
6	Intact cells	2	3400	2340
7	Intact cells	2	6500	3300
8	Intact cells	3	1880	1600

containing toluene and diphenyloxazole, 5 mg/ml, and their radioactivities determined by scintillation counting[4].

When 1-^{14}C-labeled oleic acid was incubated with rat red blood cells, an incorporation of this fatty acid into phospholipids occurred[3–5]. In the present experiments, as shown in Table I, erythrocyte membranes from both vitamin E-deficient and control rats showed an active fatty acid incorporating system. Although there was occasionally a tendency towards greater incorporation in cells of vitamin E-deficient animals, no consistent difference could be found between the erythrocytes of α-tocopherol-deprived animals and those of rats supplemented with this vitamin. The overall radioactive uptake is not significantly changed whether isolated membranes (ghosts) or intact cells were incubated.

A similar set of 7 experiments was performed using whole blood incubated with radioactive fatty acid, without any added cofactor. The incorporation of ^{14}C-labeled fatty acid per μg of phospholipid phosphorus was about 1/4 of the value obtained when the same volume of cells was suspended in the above described medium, due

TABLE II

DIFFERENTIAL INCORPORATION OF [1-^{14}C]OLEIC ACID INTO PHOSPHOLIPIDS OF RAT ERYTHROCYTE MEMBRANES

Experimental conditions as indicated in text.

Phospholipid	*Radioactivity incorporated (counts/min per μg P)*	
	E-deficient	*Control*
Lysolecithin	20	14
Sphingomyelin	21	42
Phosphatidylcholine	1530	1570
Phosphatidylserine	52	40
Phosphatidylethanolamine	242	276

probably either to dilution of the labeled fatty acid in the free fatty acid pool present in plasma or lack of cofactors needed for the reaction. Nevertheless the results (not shown) displayed the same pattern as that obtained with isolated erythrocytes reported above in Table I.

Table II shows a typical distribution of incorporation of the labeled oleic acid into individual phospholipids. Over 80% of the radioactivity found in the total phospholipid fraction was incorporated into phosphatidylcholine, the major component of the phosphoglyceride fraction in rat red blood cell membranes[9]. The same pattern of labeling was found with cells from either tocopherol-deficient or supplemented rats. The phospholipid content was also the same for both.

Very little incorporation (less and 0.1 %) of [^{14}C]oleic acid was observed in the neutral lipid fraction and no difference was observed between experimental and control animals. The control but not the experimental animals show the presence of α-tocopherol in their lipids (neutral lipid fraction), as determined by thin-layer chromatography developed in benzene, against a standard of pure α-tocopherol and detected by α,α'-dipyridyl reagent[10].

It was of interest to determine whether dialuric acid itself (in concentration known to be hemolytic in vitamin E-deficient cells)[7] induced some changes in the turnover rate of fatty acids into phospholipids in vitamin E-deficient animals. As shown in Table III, the presence of absence of dialuric acid in the incubation mixture had no significant effect on the incorporation in either control of vitamin E-deficient cells. This result was the same regardless of whether vitamin E was added to the incubation mixture or not (Table III).

TABLE III

EFFECT OF α-TOCOPHEROL OR DIALURIC ACID ON INCORPORATION OF [^{14}C]OLEIC ACID INTO PHOSPHOLIPIDS ON RED BLOOD CELL MEMBRANES

Experimental conditions as indicated in text. Dialuric acid (7 μmoles in 0.1 ml potassium phosphate buffer, 150 μmoles) and α-tocopherol (10 μmoles in 0.1 ml bovine serum albumin, 3 mg/ml) were included, as indicated, in the reaction mixture at zero time.

Expt. No.	*Cells*	*Dialuric acid, 7 μmoles*	*α-Tocopherol, 10 μmoles*	*Radioactivity incorporated (counts/min per μg P)*	
				E-deficient	*Control*
1	Ghosts	−	−	519	233
		+	−	474	263
2	Intact cells	−	−	481	417
		−	+	139	612
3	Intact cells	−	−	2410	1830
		+	−	1850	1830
4	Intact cells	−	−	3400	2340
		+	−	2600	1880
		−	+	2750	1300
		+	+	3200	1330
5	Intact cells	−	−	1200	1120
		+	−	1240	1170
		−	+	1245	1650
		+	+	1110	1230

The results of this study indicate a lack of correlation of membrane fragility (*viz.* susceptibility to hemolysis by dialuric acid) in vitamin E-deficient red blood cells and their ability to incorporate fatty acid in the phospholipid moiety. These findings, however, do not exclude the possibility of other aspects of lipid metabolism being affected in the absence of the vitamin.

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