Diet-induced Alterations in the Discoid Shape and Phospholipid Fatty Acid Compositions of Rat Erythrocytes

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ABSTRACT

For eight weeks young male rats were fed diets rich in 18:2 (stock diet, or 10% corn oil, CO) or those devoid of 18:2 (fat free, FF, or 10% hydrogenated coconut oil, HCNO). The CO and HCNO diets were fed in the absence or presence of eicosa-5,8,11,14-tetraynoic acid (TYA). When 18:2 was excluded, an increase in the level of 16:1, 18:1 and 20:3 and a decrease in 18:2 was observed in the fatty acids of red cells. On feeding TYA, an increase in 18:2 and in the case of the HCNO+TYA diet, a decrease of 12:0 and 14:0 was also observed. In all cases the levels of 20:4 in erythrocyte fatty acids were similar. Saturated fatty acids were predominant in phosphatidyl choline (PC), lysophosphatidyl-choline, (LPC) and sphingomyelin whereas unsaturated acids were predominant in phosphatidyl ethanolamine (PE), (PS), and phosphatidyl inositol (PI). Acids containing three or more double bonds comprised about 90% of the total acids in PI. In all the phospholipids, the characteristic changes in the composition of fatty acids were observed due to the exclusion of 18:2 from the diet. However, changes due to the feeding of TYA were found only in PC and LPC. In rats fed the 18:2-rich diet, about 60% of the red cells were discocytes. In those fed the 18:2-free diet, the level of discocytes decreased to about 23%, and the levels of echinocytes II and III increased. The exclusion of 18:2 for even a few days decreased the proportion of discocytes. The loss of discoid shape was reversed in a few days by feeding an 18:2-rich diet. Fatty acid analysis of erythrocytes of rats on the various dietary manipulations showed that the change in the proportion of discocytes followed the change in the level of 18:2.

INTRODUCTION

Several investigations have reported the fatty acid composition of total lipids of rat erythrocytes (1). However, to our knowledge, neither the composition of fatty acids in their individual phospholipids nor their alterations due to diet fat have been reported. In the present study, we determined the fatty acid compositions of erythrocyte phospholipids of rats which were fed various diets that are known to affect the tissue levels of 18:2 and 20:4. Furthermore, we examined the morphological structure of red cells using scanning electron microscopy and observed the importance of dietary 18:2 for the normal discoid shape of erythrocytes. A preliminary report of this study has already appeared (2).

MATERIALS AND METHODS

One month old Sprague Dawley male rats (100 g) were obtained from Hilltop Animal Supplier, Chatsworth, CA. They were divided into six groups of four each. Each group was fed for eight weeks one of the following six diets: 1) stock diet (Wayne Lab Blox; Allied Mills, Chicago, IL) which contained 4.5% fat and 45.5% 18:2 in the total fatty acids; 2) a high carbohydrate, 10% Mazola Corn Oil (CO) diet; 3) a CO diet containing 0.033% eicosa-5,8,11,14-tetraynoic acid (TYA); 4) a high carbohydrate, 10% hydrogenated coconut oil (HCNO) diet; 5) a HCNO diet containing 0.05% TYA, and 6) a high carbohydrate fat free (FF) diet. The composition of the CO, HCNO and FF diets was given previously (3).

In some experiments, rats (100 g) were fed for a week a stock diet and fed for three, five and seven days a FF diet. A group of rats which were fed the FF diet for five days were then fed the CO diet for three or seven days.

Hydrogenated coconut oil (Cobee 92) was a generous gift from PVO International, Inc., Richmond, CA. The fatty acid methyl ester standards were obtained from Applied Science Laboratories, State College, PA, and Supelco, Bellefonte, PA.

Rats were anesthetised by an intraperitoneal injection of sodium pentobarbital (50mg/ml/300 g rat) and exsanguinated using heparin-washed syringes and needles. Whole blood was transferred without pressure into heparinized vacutainer tubes. Immediately after the blood was obtained, a 0.5 ml sample was fixed in 0.5% glutaraldehyde in standard incubation mixture (SIM), which contained NaCl (141 mM), KCl (10 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1.3 mM), NaH_2PO_4 (0.8 mM) and Na_2HPO_4 (5 mM).

Whole blood was centrifuged at about 22 C at 2500 rpm for 7 min, and the plasma and buffy layer were removed. Red cells were

washed three times by suspension in SIM. Hemoglobin determinations were carried out using the cyanmethemoglobin method. The packed red cell volumes were measured in duplicate with a Phillips-Drucker hematocrit centrifuge and read on an IEC microcapillary reader.

The fresh blood samples fixed in glutaraldehyde were washed three times in SIM, sedimented on glass slides, dehydrated in a graded alcohol series and critical point dried (4). After being coated with gold-palladium, they were examined and classified using an ETEC Autoscan scanning electron microscope (5).

Total lipids from erythrocytes were extracted as described by Rose and Oklander (6). Lipid extracts were protected from the oxidation of unsaturated fatty acids by adding butylated hydroxytoluene (BHT) (0.02%) and storing under N_2. During the gas liquid chromatographic (GLC) separation of methyl esters, BHT elutes with methyl myristate. Since the HCNO diet contains 14:0, BHT was not added during the analysis of the total fatty acid composition of red cells. We have found that the absence of BHT in the lipid extracts did not reduce the level of polyunsaturated fatty acids. In the solvents used for the separation of phospholipids and methyl esters by thin layer chromatography (TLC), BHT was added. Therefore, the values for 14:0 were not obtained from the analysis of phosphoglycerides.

Phospholipids were separated by TLC, their fatty acids were converted to methyl esters and purified from dimethylacetal derivatives as described by Pullarkat et al. (7,8). Analysis of fatty acids by GLC was carried out at 180 C in a Varian Aerograph Model 2740 using a flame ionization detector and a stainless steel column (6' x 1/8") packed with 5% diethylene glycol succinate on H/P Chromosorb G. Analysis of the methyl esters from red cells of rats fed the HCNO diet was carried out at 150-180 C at 10 C/min. Areas of peaks and percent composition of fatty acid methyl esters were computed using a Varian Chromatography Data System.

RESULTS AND DISCUSSION

In the erythrocyte lipids from various species, 20:4 is a major fatty acid. In rats maintained on linoleate-rich diets, as much as 30% of the total fatty acids in red cells is 20:4 (9). In the present study, rats were fed various diets that influence the tissue levels of 18:2 and 20:4. These were diets rich in 18:2, free of 18:2 and those which contained TYA – an inhibitor of the synthesis of 20:4 from 18:2 (10,11).

When 18:2 was excluded from the diets, the rat growth was somewhat depressed. Rats fed stock and CO diets for 8 weeks weighed about 527 g and 512 g, respectively, while those fed the HCNO and FF diets weighed 481 g and 479 g, respectively Dietary TYA caused a further reduction in their weights. Rats fed the CO + TYA diet, weighed 486 g while those fed the HCNO + TYA diet, weighed 400 g. The blood samples of all rats had a similar packed red cell volume (43.0 – 44.9%) and hemoglobin content (15.1 – 15.7g%). Since the amount of blood in rats has been estimated to be 7.6 ± 0.2 ml/100 g body weight (12), it would appear that it is somewhat decreased by the exclusion of 18:2 or the inclusion of TYA in the diet.

As observed previously (9,13), the composition of total fatty acids in rat erythrocytes was altered when 18:2 was omitted from the diet (Table I). The 16:1 and 20:3 levels and the values for the ratio 18:1/18:0 were enhanced while the levels of 18:2 were decreased. In the present study, the 20:4 level in the total fatty acids of erythrocytes was not decreased. A marked reduction in the level of 20:4 has been reported in red cells when weanling rats were fed an 18:2 deficient diet for 6 months (13). When TYA was included in the diet, a significant decrease in values for the ratios 16:1/16:0 and 18:1/18:0 has been observed in the lipids of liver and plasma of rats (14). In the erythrocyte lipids, such a decrease in the relative levels of monounsaturated fatty acids was small (Table I). As in the case of the liver and plasma lipids (14), when HCNO+TYA diet was fed, the levels of 12:0 and 14:0 were decreased in the erythrocyte lipids (Table I). As previously observed with several other tissues (10,11,14), the level of 18:2 was increased and the proportion of 20:4 to 18:2 was decreased in erythrocytes due to dietary TYA.

A detailed study of the fatty acid composition of individual phospholipids of rat erythrocytes has not been carried out (1). In some previous investigations, not all the phospholipid species were analyzed, while in others several fatty acids were grouped together (1). We have determined the fatty acid compositions of various individual phospholipids in erythrocytes of rats and their changes due to different diets (Table II–VII). Each phospholipid species exhibited a characteristic fatty acid composition which was altered by the dietary manipulations. The phosphatidyl choline (PC), lysophosphatidyl choline (LPC), and sphingomyelin contained relatively more saturated acids. In PC and LPC, 16:0 and 18:0 were predominant, while in sphingomyelin, 16:0 and 24:0 were major saturated acids (Table II–IV). On the

TABLE I

Fatty Acid Composition of Erythrocytes of Rats Fed Different Diets[a]

Fatty acid	Stock diet[b]	CO diet[b]		HCNO diet[b]		FF diet
		-TYA	+ TYA	-TYA	+TYA	
12:0	0	0	0	0.7 ± 0.1	0.3 ± 0.05	0
14:0	0.7 ± 0.1	0.8 ± 0.07	T	1.5 ± 0.1	0.6 ± 0.04	0.6 ± 0.05
16:0	18.9 ± 1.3	16.4 ± 0.3	18.5 ± 0.3	19.4 ± 0.5	19.3 ± 0.5	20.0 ± 0.5
16:1	0.7 ± 0.1	T	T	2.4 ± 0.1	1.6 ± 0.04	2.6± 0.1
18:0	21.0 ± 1.2	21.3 ± 0.4	21.2 ± 0.3	17.9 ± 0.2	19.3 ± 0.3	19.1± 0.2
18:1	10.6 ± 0.6	9.5 ± 0.3	8.2 ± 0.2	13.8 ± 0.2	13.3 ± 0.3	16.2± 0.4
18:2	10.3 ± 0.5	12.5 ± 0.2	18.9 ± 0.5	1.6 ± 0.1	3.6 ± 0.1	1.6± 0.1
18:3	T	T	T	---	---	---
20:3W9	---	---	---	7.2 ± 0.3	7.5 ± 0.3	6.2± 0.2
22:0	T	T	T	1.1 ± 0.1	3.1 ± 0.1	1.0± 0.1
20:4	26.5 ± 1.0	28.8 ± 0.3	26.1 ± 0.4	28.0 ± 0.3	26.4 ± 0.7	27.8± 0.6
22:4[c]	4.6 ± 0.6	5.6 ± 0.2	4.2 ± 0.2	3.3 ± 0.2	2.5 ± 0.2	2.7± 0.1
22:5	1.9 ± 0.3	1.7 ± 0.1	0.8 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	0.8± 0.2
22:6	3.9 ± 0.5	3.3 0.1	1.3 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.6± 0.1
16:1/16:0	---	---	---	0.12 ± 0.01	0.08 ± 0.003	0.13± 0.004
18:1/18:0	0.51 ±0.02	0.45 ± 0.02	0.38 ± 0.02	0.77 ± 0.01	0.69 ± 0.02	0.85 ±0.02
20:4/18:2	2.57 ± 0.19	2.3 ± 0.06	1.4 ± 0.04	17.7 ± 0.9	7.3 ± 0.3	18.1 ±0.8

[a]Percent of total fatty acids is given as Mean ± SE of analysis with erythrocytes from four rats in each diet group. Duplicate determinations were carried out with erythrocytes from each rat. Values 0.5% or less are given as T.

[b]The stock diet contained the fatty acids: 14:0, 3.3%; 16:0, 18.2%; 16:1, 2.5%; 18:0, 4.1%; 18:1, 23.3%, 18:2, 45.5% and 18:3, 3.1%. The corn oil (CO) diet contained the fatty acids: 16:0, 10.7%; 18:0, 1.8%; 18:1, 24.8%; 18:2, 61.9%; and 18:3, 1.0%. The hydrogenated coconut oil (HCNO) diet contained the fatty acids: 6:0, 1.0%; 8:0, 11.9%; 10:0, 11.9%; 12:0, 18.6%; 14:0, 20.9%; 16:0, 16.7%; 18:0, 18.6%, and 18:1, 0.4%.

[c] Included 24:0 and 24:1.

TABLE II

Fatty Acid Composition of Phosphatidylcholine from Erythrocytes of Rats Fed Different Diets[a]

Fatty acid	Stock diet	CO diet		HCNO diet		FF diet
		-TYA	+TYA	-TYA	+TYA	
16:0	35.6 ± 2.2	40.6 ± 0.4	37.4 ± 0.7	35.4 ± 0.6	34.0 ± 0.8	36.8 ± 1.2
16:1	0.9 ± 0.1	T	T	3.7 ± 0.2	3.0 ± 0.1	3.8 ± 0.5
18:0	27.6 ± 1.8	20.3 ± 0.8	21.1 ± 1.0	18.2 ± 0.8	20.4 ± 0.6	15.4 ± 0.4
18:1	8.3 ± 0.5	7.1 ± 0.5	6.8 ± 0.7	24.5 ± 0.8	22.0 ± 1.0	24.3 ± 0.8
18:2	12.3 ± 0.6	15.0 ± 0.5	20.3 ± 0.3	1.5 ± 0.1	5.8 ± 0.2	1.4 ± 0.2
18:3	1.1 ± 0.2	T	T	---	---	---
20:3W9	---	---	---	8.0 ± 0.5	7.5 ± 0.3	9.1 ± 0.8
20:4	13.8 ± 0.6	17.2 ± 1.6	13.2 ± 0.4	8.6 ± 0.6	7.3 ± 0.4	9.3 ± 0.5

[a]Percent of total fatty acids given are Mean ± SE of values from three determinations with PC isolated from red cell lipids of separate rats in each diet group.

other hand, in phosphatidyl ethanolamine (PE), phosphatidyl serine (PS) and phosphatidyl inositol (PI), unsaturated acids were present in relatively large levels. In these lipids the 20:4 level varied from 42 to 60%. The level of 18:0 was greater in PS than in PE or PI. The composition of PI was unique in that acids containing three or more double bonds comprised about 90% of the total fatty acids.

The fatty acid compositions of various phospholipids were altered due to the dietary manipulations. When 18:2 was omitted from the diet, 16:1 and 20:3 levels were increased and the 18:2 and 20:4 levels were decreased in PC and LPC. In these lipids relatively more 18:1 was present than 18:0. As observed by Watson previously (15), relatively more of 24:1 than 24:0 was found in the sphingomyelin fraction of erythrocyte lipids of rats fed the 18:2-free diets (Table IV). Upon feeding the

TABLE III

Fatty Acid Composition of Lysophosphatidyl Choline from Erythrocytes of Rats Fed Different Diets[a]

Fatty acid	Stock diet	CO diet		HCNO diet		FF diet
		-TYA	+TYA	-TYA	+TYA	
16:0	33.1	39.0	37.1	36.9	39.9	35.8
16:1	1.4	1.1	0.5	3.7	2.4	3.2
18:0	40.6	29.8	36.2	24.2	28.6	24.9
18:1	8.1	11.9	8.4	20.4	15.7	21.8
18:2	6.9	6.7	9.3	T	T	T
18:3	1.4	T	T	---	---	---
20:3W9	---	---	---	7.6	6.4	6.1
20:4	7.9	11.2	8.4	7.2	6.8	8.2

[a]Percent of total fatty acids given are means of closely agreeing values from duplicate determinations with the pooled lysophosphatidyl choline fractions of red cell lipids in each dietary condition.

TABLE IV

Fatty Acid Composition of Sphingomyelin from Erythrocytes of Rats Fed Different Diets[a]

Fatty acid	Stock diet	CO diet		HCNO diet		FF diets
		-TYA	+ TYA	-TYA	+TYA	
16:0	30.5 ± 2.1	26.1 ± 2.8	26.4 ± 3.6	30.1 ± 1.3	27.6 ± 1.8	28.0 ± 1.5
18:0	7.3 ± 0.6	7.1 ± 1.1	6.7 ± 0.8	6.0 ± 0.7	6.9 ± 1.0	7.6 ± 0.6
18:1	8.0 ± 0.8	6.0 ± 0.7	6.1 ± 0.4	3.9 ± 0.2	2.5 ± 0.5	6.3 ± 0.2
20:0	1.5 ± 0.2	2.1 ± 0.4	1.7 ± 0.5	2.2 ± 0.4	1.4 ± 0.2	2.0 ± 0.4
22:0[b]	7.9 ± 0.7	8.2 ± 0.8	9.2 ±1.0	9.1 ± 0.8	8.6 ± 0.5	8.0 ±0.5
24:0	25.9 ± 0.8	28.9 ± 2.7	28.7 ± 1.3	17.1 ± 0.7	18.3 ±0.5	15.9 ± 0.6
24:1	11.3 ± 0.6	12.6 ± 0.8	13.7 ± 0.6	30.3 ± 0.4	33.2 ± 1.3	30.7 ± 1.6
24:2	7.2 ± 1.1	8.8 ± 0.7	7.3 ± 0.9	1.1 ± 0.2	1.3 ± 0.2	1.3 ± 0.2

[a]Percent of total fatty acids given are Mean ± SE of values from three determinations with Sphingomyelin isolated from red cell lipids from separate rats in each group.

[b]Contained small amounts of 22:1.

TABLE V

Fatty Acid Composition of Phosphatidyl Ethanolamine From Erythrocytes of Rats Fed Different Diets[a]

Fatty acid	Stock diet	CO diet		HCNO diet		FF diet
		-TYA	+ TYA	-TYA	+ TYA	
16:0	8.9 ± 0.6	9.6 ± 0.5	12.2 ± 1.2	9.1 ± 0.4	9.1 ± 0.7	11.5 ± 0.8
16:1	T	T	T	2.5 ± 0.1	2.0 ± 0.1	2.5 ± 0.2
18:0	7.5 ± 0.5	9.0 ± 0.7	12.4 ± 0.9	10.8 ± 0.2	13.5 ± 0.5	8.8 ± 0.5
18:1	14.5 ± 1.5	17.4 ± 1.6	15.6 ± 0.5	17.6 ± 0.1	15.1 ± 0.9	20.1 ± 0.4
18:2	8.7 ± 0.5	10.6 ± 0.5	10.3 ± 0.4	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
18:3	T	T	T	---	---	---
20:3W9	---	---	---	13.8 ± 0.8	13.6 ± 0.3	10.6 ± 0.8
20:4	52.4 ± 2.2	46.6 ± 1.0	44.9 ± 1.1	43.2 ± 0.5	42.4 ± 0.8	42.0 ± 1.2
22:4	4.0 ± 0.2	3.7 ± 1.1	2.9 ± 0.1	1.6 ± 0.2	1.8 ± 0.4	2.3 ± 0.1
22:5	1.6 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.2
22:6	1.8 ± 0.1	1.9 ± 0.1	0.9 ±0.2	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.2

[a]Percent of total fatty acids given are Mean ± SE of values from three determinations with PE isolated from red cell lipids of separate rats in each diet group.

diets devoid of 18:2, the level of 18:2 decreased and of 20:3 increased in PE, PS and PI. However, only in PI did the relative level of 18:1 to 18:0 increase. Dietary TYA did not alter the fatty acid compositions of sphingomyelin, PE, PS and PI, but did cause an increase in the level of 18:2 in PC and LPC. Earlier studies with red cell ghosts have shown that all

TABLE VI

Fatty Acid Composition of Phosphatidyl Serine from Erythrocytes of Rats Fed Different Diets[a]

Fatty acid	Stock diet	CO diet		HCNO diet		FF diet
		-TYA	+ TYA	-TYA	+TYA	
16:0	6.1 ± 0.4	6.0 ± 0.4	5.6 ± 0.4	6.5 ± 0.6	7.4 ± 0.7	6.2 ± 0.2
16:1	T	T	T	0.8 ± 0.1	T	T
18:0	29.6 ± 2.6	26.3 ± 0.4	27.9 ± 0.8	24.4 ± 0.7	25.3 ± 1.2	22.7 ± 1.0
18:1	6.5 ± 0.5	6.3 ± 0.5	6.5 ± 0.4	9.5 ± 0.4	8.3 ± 0.5	10.4 ± 0.9
18:2	3.7 ± 0.4	7.2 ± 0.9	6.9 ± 0.9	T	T	T
18:3	T	T	T	---	---	---
20:3W9	---	---	---	7.2 ± 0.8	7.7 ± 0.5	6.6 ± 0.6
20:4	50.4 ± 2.4	51.3 ± 0.8	50.7 ± 1.3	49.1 ± 0.9	49.7 ± 0.6	50.1 ± 1.3
22:4	1.7 ± 0.2	1.1 ± 0.1	1.1 ± 0.2	1.0 ± 0.2	0.9 ± 0.1	1.4 ± 0.2
22:5	1.7 ± 0.3	1.5 ± 0.2	1.1 ± 0.2	1.4 ± 0.3	T	1.7 ± 0.1

[a]Percent of total fatty acids given are Mean ± SE of values from three determinations with PS isolated from red cell lipids from red cell lipids from separate rats in each diet group.

TABLE VII

Fatty Acid Composition of Phosphatidyl Inositol from Erythrocytes of Rats Fed Different Diets[a]

Fatty acid	Stock diet	CO diet		HCNO diet		FF diet
		-TYA	+ TYA	-TYA	+ TYA	
16:0	3.4 ± 0.4	4.3 ± 0.4	3.8 ± 0.5	3.6 ± 0.3	3.4 ± 0.5	3.9 ± 0.4
16:1	T	T	T	0.8 ± 0.1	T	0.8 ± 0.1
18:0	2.1 ± 0.5	1.6 ± 0.2	1.1 ± 0.1	1.3 ± 0.2	1.7 ± 0.2	1.4 ± 0.2
18:1	2.3 ± 0.4	1.9 ± 0.2	1.2 ± 0.1	4.4 ± 0.4	3.8 ± 0.3	4.9 ± 0.4
18:2	2.0 ± 0.6	1.6 ± 0.1	1.4 ± 0.4	---	---	---
18:3	0.8 ± 0.1	T	T	---	---	---
20:3W9	---	---	---	19.6 ± 0.6	20.2 ± 0.8	19.6 ± 0.6
20:4	51.2 ± 1.3	59.0 ± 0.8	58.8 ± 1.5	60.0 ± 1.0	59.5 ± 1.9	58.9 ± 1.0
22:4	17.7 ± 1.4	19.4 ± 0.7	20.3 ± 1.2	4.0 ± 0.2	3.8 ± 0.4	4.0 ± 0.2
22:5	9.6 ± 0.8	6.0 ± 0.6	6.1 ± 0.6	2.5 ± 0.6	2.9 ± 0.1	2.7 ± 0.1
22:6	10.5 ± 0.4	6.2 ± 0.2	7.1 ± 0.5	3.9 ± 0.1	4.5 ± 0.4	3.6 ± 0.4

[a]Percent of total fatty acids given are Mean ± SE of values from three determinations with PI isolated from red cell lipids from separate rats in each diet group.

TABLE VIII

Morphologic Structure of Erythrocytes of Rats Fed Different Diets[a]

Cell type	Stock diet	CO diet		HCNO diet		FF diet
		-TYA	+ TYA	-TYA	+ TYA	
Discocytes	63.9 ± 0.6	56.8 ± 4.2	57.7 ± 4.7	23.5 ± 4.4	27.6 ± 3.5	16.9 ± 1.5
Knizocytes	---	0.3 ± 0.2	2.1 ± 0.8	---	---	0.3 ± 0.2
Stomatocytes	7.3 ± 0.3	4.0 ± 1.1	4.0 ± 0.6	1.3 ± 0.6	2.4 ± 0.5	1.9 ± 0.6
Echinocytes I	25.8 ± 0.5	31.1 ± 4.6	27.9 ± 2.3	32.6 ± 2.1	33.6 ± 4.7	44.2 ± 3.0
Echinocytes II	2.4 ± 0.2	7.0 ± 0.1	7.6 ± 2.8	34.9 ± 4.7	30.1 ± 3.7	30.7 ± 4.4
Echinocytes III	---	0.7 ± 0.3	0.6 ± 0.2	5.9 ± 1.0	4.4 ± 1.0	5.6 ± 1.5

[a]Percent distribution of the various cell types are given as Mean ± SE of the values obtained by counting 500 cells in duplicate from the blood sample from each rat in the diet group. Samples of blood from four rats in the CO and FF diet groups and three rats in the HCNO and stock diet groups were used.

the lipid present in the red cell resides in the membrane (16,17). Thus, the composition of fatty acids and their changes that were observed in our investigation using red cells must be those of the membranes.

Although the level of 20:4 in the total fatty acids of erythrocytes was essentially similar under various dietary conditions (Table I), exclusion of 18:2 or addition of TYA in the diet resulted in a decrease in the level of 20:4 in

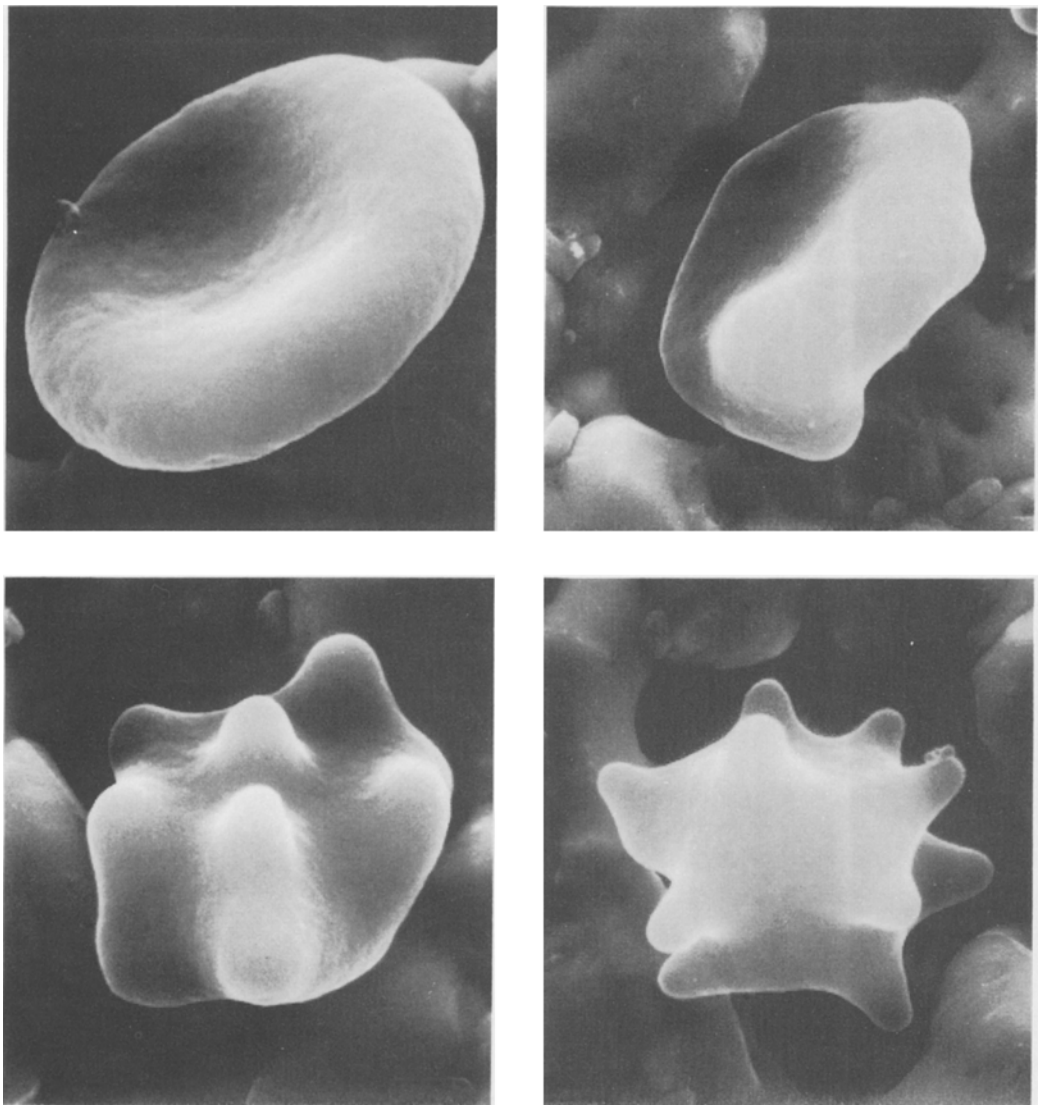

FIG. 1. Scanning electron microscopic examination of rat erythrocytes. Top left, Discocyte (X12,00); top right, Echinocyte I (X13,000); bottom left, Echinocyte II (X13,000); bottom right, Echinocyte III (X13,000).

PC and LPC (Table II, III). Whether this discrepancy is due to the change in the content of individual phospholipids due to diets is not known at the present time. Previously, Farnsworth et al. observed that the relative amount of 'lecithin' increased and 'cephalin' decreased in erythrocytes of rats which were fed a fat free diet as compared to their controls (18). On the other hand, De Gier and Van Deenen (19) reported that the variation in the regimen of rats did not significantly change the proportions of major phospholipids.

Red cells from rats fed an 18:2-deficient diet for 3 months were reported to be morphologically normal (15). However, this conclusion was based on the results of cell sizing with a Coulter Counter. In our studies of the morphology of the erythrocytes using scanning electron micriscopy, alteration of the normal biconcave structure of the discocytes was observed due to the exclusion of 18:2 from the diet (Table VIII). A majority of the cells (60%) from rats fed linoleate-rich diets (stock diet, CO, CO+TYA) are discocytes which are characterized by their biconcave structure, while about 30% of cells are irregularly contoured

TABLE IX

Effect of Exclusion or Addition of Linoleate to Diets Fed to Rats for Short Periods on the Morphologic Structure of Erythrocytes[a]

	Number of days on FF diet[b]			Number of days on CO diet			
Cell type	0	3	7	0[c]	3[c]	7[c]	7[d]
Discocytes	66.4	43.0	9.8	23.2	41.0	63.2	66.8
Knizocytes	6.4	3.8	0.4	1.2	0.0	3.4	0.8
Stomatocytes	15.6	6.1	2.2	4.0	5.9	4.4	3.6
Echinocytes I	11.6	31.9	31.2	32.0	23.4	17.8	20.4
Echinocytes II	0.0	14.5	44.0	36.8	26.8	9.3	7.2
Echinocytes III	0.0	0.6	11.8	2.8	2.9	1.8	0.8

[a]Percent of various types of cells given is mean of values from counting 500 cells in duplicate with samples of blood pooled from two young rats or separate samples from adult rats.

[b]Young rats (100 g) fed a stock diet were then fed a FF diet as indicated.

[c]Young rats fed a stock diet were fed FF diet for five days and then the CO diet as indicated.

[d]Young rats were fed a FF diet for eight weeks and then fed the CO diet as indicated.

TABLE X

Changes in the Fatty Acid Composition of Erythrocytes due to the Exclusion or Addition of Linoleate to Diets Fed to Rats for Short Periods[a]

	Number of days on FF diet			Number of days on CO diet			
Fatty acid	0	3	7	0	3	7	7
16:0	26.2	26.6	27.5	27.7	27.3	28.0	26.1
16:1	T	0.7	1.9	2.1	0.8	0.6	0.5
18:0	15.4	14.0	14.8	13.2	15.2	13.6	16.4
18:1	9.1	12.4	16.5	14.3	13.7	10.2	10.7
18:2	12.5	6.8	2.1	4.4	6.6	10.8	8.3
20:3w9	---	---	2.8	1.2	0.5	0.5	3.1
22:0	T	T	T	T	T	T	0.5
20:4	28.8	28.4	26.9	26.2	25.5	27.2	27.2
24:0)							
22:4)	3.4	4.8	4.6	4.1	5.4	4.8	4.0
24:1)							
22:5	1.2	1.9	1.7	2.9	2.0	2.1	1.2
22:6	3.2	3.8	1.4	3.5	2.7	2.1	1.8

[a]Percent of total fatty acids given are the means of closely agreeing values from duplicate determinations with lipid extracts of erythrocytes of rats used in Table IX. Values 0.5% or less are given as T.

discocytes (Echinocytes I) (Fig. 1). Flat red cells with spicules (Echinocytes II) are few 2-7%), and spherical cells with spicules (echinocytes III) are practically absent (Table VIII, Fig. 1). However, when rats were fed diets devoid of 18:2 (HCNO, HCNO + TYA, FF) the proportion of discocytes decreased to 17-28%, while echinocytes II and echinocytes III increased to 35% and 6%, respectively. These structural changes appear to be associated with the presence of 20:3 and the reduced level of 18:2 in red cells (Table I).

In order to establish the involvement of dietary 18:2 in maintaining the normal structure of erythrocytes, we determined whether the loss of discoid structure could be reversed by feeding a 18:2-rich diet (Table IX). Furthermore, since the life span of red cells is about 60 days, it was not known whether the increased level of echinocytes produced by feeding rats a diet devoid of 18:2 for eight weeks was related to the turnover of the cells. Hence, we determined the fatty acid composition and morphological structure of red cells from rats which were fed a FF diet for only a few days and also from those which were fed a FF diet and then fed a diet rich in 18:2 for a few days (Table IX,X).

A majority of the red cells (66%) in rats fed the stock diet were discocytes. Echinocytes I were few (12%) and echinocytes II and III were practically absent (Table IX, Fig. 2). On feeding

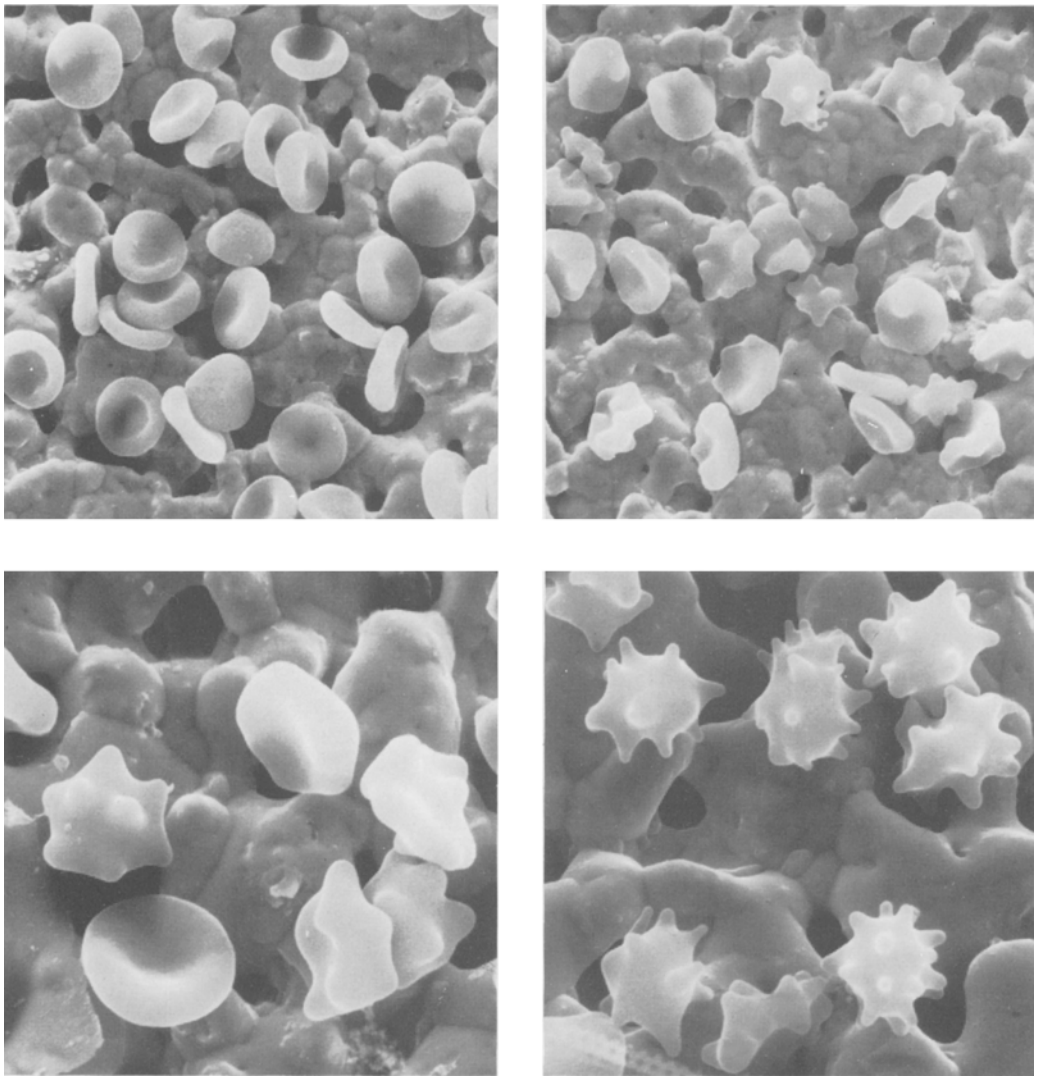

FIG. 2. Scanning electron microscopic examination of erythrocytes from rats fed 18:2-rich or 18:2-free diets. Top left, Erythrocytes were obtained from rats maintained on a stock diet; top right, bottom left and right, erythrocytes were from rats fed a FF diet for one week. Top, X2500 Bottom, X5000.

a FF diet for one week, the level of discocytes decreased to 10%, while the level of echinocytes I, II and III increased to 31%, 44% and 12%, respectively (Table IX, Fig. 2). Even after three days, a significant reduction in discocytes and an increase in echinocytes was observed (Table IX). Thus, in order for the structural changes in erythrocytes to occur, a long term (eight weeks) feeding of an 18:2-deficient diet is not needed.

When rats were fed a FF diet for five days or eight weeks and then fed a CO diet for a week, a reversal in the loss of discocytes occurred (Table IX). An appreciable increase in the number of discocytes and a decrease in echinocytes I and II was found by feeding the CO diet for even three days. Thus, loss of the discoid shape of erythrocytes due to feeding rats an 18:2-free diet is reversed by feeding them an 18:2-rich diet. Since structural changes have occurred in erythrocytes by short term dietary manipulations, it is clear that these changes are not related to the life span of red cells.

In erythrocyte lipids the characteristic changes in the composition of fatty acids due to feeding a FF diet, (presence of 20:3; decrease of 18:2 and relatively high levels of 16:1 and 18:1) were observed in a few days (Table

X). The changes in the composition due to feeding CO diet (increased levels of 18:2; low levels of 20:3; and a decrease in 16:1 and 18:1) also occurred (Table X).

In rats fed a FF diet for eight weeks, the erythrocyte fatty acids contained significant levels (6.2%) of 20:3 and very low levels (1.6% of 18:2 (Table I). When they were then fed a CO diet for one week, their red cells still contained 20:3 (3.1%) while the level of 18:2 increased to 8.3% (Table X). Since the discocyte level was normal (Table IX), it is apparent that the presence of 20:3 may not be related to the loss of discocytes or increase in the number of echinocytes. On the other hand, the loss of discocytes and the concomitant increase in the number of echinocytes is related to the level of 18:2 in erythrocyte fatty acids (Table X). However, it cannot be concluded from these results that the alterations in the composition of fatty acids are responsible for the structural changes of the red cells. Exposure of erythrocytes to plasma which contains free fatty acids or LPC or changes in the ratio of cholesterol to phospholipids in erythrocytes alters the structure of red cells (20,21). Whether the structural modifications seen in our study are due to such changes in the lipid composition of plasma or red cells is not yet known. However, our results do demonstrate the importance of dietary linoleate in maintaining the normal discoid shape of red cells.

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