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# The Effect of Intravenous Hyperalimentation on Erythrocyte Lipids (37861)

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(Introduced by M. F. La Via)

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Essential fatty-acid deficiency has been studied in several animals, some microorganisms, insects, and the human infant (1). In the adult man, essential fatty-acid deficiency has been produced only with difficulty for reasons that have been discussed in detail elsewhere (2). Recently, however, it has been shown that the introduction of intravenous nutrition or intravenous hyperalimentation (3) as a therapy in gastrointestinal disorders and malnutrition resulted in the appearance of essential fatty-acid deficiency in plasma lipids (4, 5).

The experiments to be reported herein describe the effect of intravenous hyperalimentation on human erythrocyte lipids.

*Materials and Methods.* Each of the four patients had been studied previously when the effect of intravenous hyperalimentation on plasma lipids was investigated (unpublished data). Controls were two volunteers from the medical staff. The intravenous therapy consisted of daily intake of 3 liters of liquid (24 hr infusion) having the composition given in Table I.

Blood samples were collected during the infusion in EDTA and centrifuged at 4° at 1000*g* for 10 min to remove the plasma. The cells were washed three times with 2 vol of 0.15 *M* NaCl in order to remove leucocytes and platelets. Red cells were suspended in physiological saline to a hematocrit of 65–75%. Aliquots were taken for hematocrit determination, and red cells were enumerated in a Coulter Model B electronic particle counter. Red cells were always extracted immediately after the final wash with 2:1 (v/v) chloroform–methanol as described by Nelson (6). Plasma lipids were extracted similarly. The extracted lipids were washed with 0.37% KCl according to Folch *et al.* (7) and dried *in vacuo.*

Aliquots of the total lipid extract were taken, and the following determinations were carried out: Total lipid was determined gravimetrically, cholesterol was assayed according to Zlatkis *et al.* (8), and lipid phosphorus according to Bartlett (9). Phospholipids were run on thin-layer plates using silica-gel G plates (Q5; Quantum Industries, Fairfield, NJ). The solvent system was chloroform–methanol–13.5 *N* ammonia–water (70:30:4:1, v/v), and the plates were activated for 90 min at 110°. This system consistently separated ethanolamine, serine, choline phosphoglycerides, sphingomyelin, and lysolecithin; the inositol phosphoglycerides usually ran at the lower margin of sphingomyelin and when eluted, they were

TABLE I. Composition per Liter of the Infusion Fluid.

| | |
|---|---|
| Dextrose (50% solution) | 500 cc |
| Freamine[a] | 500 cc |
| Phosphorus | 400 mEq |
| $K^+$ | 40 mEq |
| $Cl^-$ | 38 mEq |
| $Na^+$ | 50 mEq |
| $Mg^{2+}$ | 16 mEq |
| $Ca^{2+}$ | 10 mEq |

[a] 8.5% Freamine solution. Approximate concentration of amino acids (g/100 ml): L-Isoleucine 0.59; L-leucine 0.77; L-lysine HCl 0.77; methionine 0.45; L-phenylalanine 0.48; L-threonine 0.34; L-tryptophan 0.13; L-valine 0.56; L-alanine 0.60; L-arginine 0.31; L-histidine 0.24; L-proline 0.95; L-serine 0.50; glycine 1.8; L-cysteine · HCl · $H_20$ < 0.02.

eluted together. For purposes of identification, phospholipid standards (Supelco, Inc., Bellefonte, PA, and Applied Sciences Laboratories, State College, PA) were run on each plate along with the unknowns. Additional plates spotted and developed in an identical manner were sprayed with ninhydrin or Dragendorff's reagent to locate amino- and choline-containing lipids. Generally, phospholipids were stained with fluorescein and were observed under uv light. The phosphorus content of individual fractions was determined, and phospholipid fatty acids were methylated and quantitated as previously described (10).

Fatty acids were identified by their retention times on polar and nonpolar solvent columns and are designated by a shorthand system in which the chain length and number of double bonds are specified; for example, linoleic acid = 18:2. Standard methyl esters purchased from the Hormel Institute were also used. 5,8,11-Eicosatrienoic acid was identified by comparison with lipids isolated from rats deficient in essential fatty acids. Thin-layer chromatography of methyl esters on silver-nitrate plates was performed as previously described (11).

In all procedures involving lipids, reagent-grade redistilled solvents were employed, and operations were carried out under nitrogen whenever possible to minimize oxidation of unsaturated acids.

The analytical procedures were reproducible. A pooled erythrocyte separation in four separate samples (1 ml) taken within 26 days after the start of the therapy gave the following relative percentage concentrations for the major fatty acids: palmitic acid, 22.0 ± 0.42; stearic acid, 14.2 ± 0.41, oleic acid, 24.4 ± 0.36; linoleic acid, 3.2 ± 0.027; and arachidonic acid, 11.0 ± 0.56 (means ± SD of four analyses).

*Results and Discussion.* Values for total lipid, total cholesterol, and total phospholipid of erythrocytes are shown in Table II. They were calculated on the basis of mg/100 ml packed cells and mg/cell. Although there is a scattering of values for total lipids, cholesterol, and phospholipids per cell, they are comparable to the normal values, suggesting that the lipid composition of the red blood cells remained unchanged during the therapy. Table III shows the distribution of individual phospholipids in red blood cells. Data are for the predominant phospholipids and are reported as the amount of individual phospholipid phosphorus divided by the total

TABLE II. Total Lipid, Total Cholesterol, and Total Phospholipid Contents of Erythrocytes of Patients on Intravenous Nutrition.

| Patient | Date | Lipid | Cholesterol | Phospholipid | Lipid | Cholesterol | Phospholipid |
|---|---|---|---|---|---|---|---|
| | | | mg/100 ml | | | mg/$10^{-10}$ cells | |
| J.M. | 4.13 | 498 | 121 | 305 | 4.98 | 1.21 | 3.05 |
| | 4.26 | 488 | 120 | 301 | 4.88 | 1.20 | 3.01 |
| | 5.3 | 502 | 126 | 298 | 5.02 | 1.26 | 2.98 |
| | 5.10 | 493 | 142 | 315 | 4.93 | 1.42 | 3.15 |
| | 5.15 | 498 | 105 | 280 | 4.98 | 1.05 | 2.80 |
| | 5.21 | 500 | 140 | 308 | 5.0 | 1.40 | 3.08 |
| | 6.25 | 508 | 131 | 302 | 5.1 | 1.30 | 3.02 |
| R.W. | 4.26 | 478 | 116 | 300 | 4.75 | 1.16 | 3.0 |
| | 5.3 | 489 | 114 | 288 | 4.89 | 1.14 | 2.88 |
| | 5.10 | 492 | 113 | 292 | 4.92 | 1.13 | 2.92 |
| | 5.17 | 485 | 118 | 305 | 4.85 | 1.18 | 3.05 |
| | 5.25 | 499 | 107 | 289 | 4.99 | 1.07 | 2.89 |
| | 5.31 | 478 | 126 | 298 | 4.78 | 1.26 | 2.98 |
| | 6.6 | 482 | 120 | 296 | 4.82 | 1.20 | 2.96 |
| Control values, range | | 460–530 | 110–140 | 250–325 | 4.6–5.3 | 1.1–1.4 | 2.5–3.2 |

TABLE III. Phospholipid Composition (% of Total Phosphorus) of Erythrocytes of Patients on Intravenous Nutrition.[a]

| Patient | Date | PE | PC | Sph | PS |
|---|---|---|---|---|---|
| J.M. | 4.13 | 26.3 | 33.1 | 26.9 | 13.8 |
| | 4.26 | 30.2 | 30.2 | 23.5 | 16.1 |
| | 5.3 | 28.8 | 30.6 | 24.4 | 16.3 |
| | 5.10 | 30.3 | 33.5 | 22.4 | 13.8 |
| | 5.15 | 28.6 | 33.3 | 22.6 | 15.5 |
| | 5.21 | 26.1 | 29.8 | 28.3 | 15.8 |
| R.W. | 4.26 | 30.7 | 28.2 | 25.6 | 15.6 |
| | 5.3 | 28.0 | 26.5 | 28.0 | 17.4 |
| | 5.10 | 28.2 | 26.0 | 28.2 | 17.6 |
| | 5.17 | 29.4 | 29.4 | 26.5 | 14.7 |
| | 5.25 | 30.2 | 29.2 | 26.0 | 14.6 |
| | 5.31 | 29.2 | 29.2 | 25.7 | 15.9 |
| | 6.6 | 27.7 | 28.6 | 26.9 | 16.8 |
| Control values, range | | 24.5–30.2 | 27.0–33.0 | 22.2–28.2 | 10.4–16.0 |

[a] Abbreviations: PE, phosphatidylethanolanine; PC, phosphatidycholine; Sph, sphingomyelin; PS, phosphatidylserine.

amount of phosphorus recovered from the chromatogram. It is shown that values were essentially unchanged during the intravenous nutrition and they remained identical to the controls. These results are in positive agreement with previous investigations in subjects on controlled diets including fat-free diets (12, 13). Other studies have also shown that erythrocyte lipid composition is not affected by elevated plasma-lipid levels (14); only in certain specific diseases like liver disorders (15) and acanthocytosis was it shown that changes in phospholipid composition took place (16, 17).

Phospholipid fatty-acid analysis as percentage by weight of total fatty acid is given in Table IV. Changes in the constituent fatty acids included a decrease by approximately 55–65% in 18:2ω6 and 20:4ω6 and a large increase in 16:0, 16:1ω7 and 18:1ω9. 5,8,11-Eicosatrienoic acid (20:3ω9) increased four-fold, and this result clearly distinguishes the present finding from previous results (18). The importance of 20:3ω9 as a sign of essential fatty-acid deficiency in red blood cells of several animals including monkeys (19) has been amply demonstrated (1, 2). In rats, when dietary linoleate was restricted, 20:3ω9 accumulated in erythrocyte lipids, comprising over 16% of the total fatty acids (20). In man, however, several studies have shown no increase of 20:3ω9 in red-cell phospholipids either after dietary manipulation (18), cases of malnutrition, or in a variety of hematological disorders (17), and this is in marked contrast to the experiments reported in the present study in which changes in the erythrocyte $C_{20}$ unsaturates did occur. Whether this is due to the high-caloric intake during the intravenous feeding (3, 5) or the subsequent hyperlipemia (4) remains to be seen.

Figure 1 presents analyses of plasma and red-cell phospholipid fatty acids before, during the intravenous infusion, and after its discontinuation when the patient was given orally regular food supplemented with safflower oil. The exact time of the discontinuation is marked, and this demonstrates that the changes in the proportion of the fatty acids occurred as a result of this treatment. Similar results (not shown) were obtained from a second patient who was subjected to a similar treatment. Figure 1 shows that corn oil with its high (77%) linoleic-acid content resulted in a rapid change in plasma fatty-acid composition. In plasma phospholipids, the concentration of linoleic and arachidonic acids increased rapidly approaching the zero-time values, whereas oleic and 20:3ω9 acids

TABLE IV. Fatty-Acid and Aldehyde Composition of Total Phospholipids from Erythrocytes of Patients on Intravenous Hyperalimentation.

| | Distribution (% of total fatty acid and aldehyde) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | J.M.[a] | | | | | | R.W.[a] | | | | | | |
| Component | 4.13 | 4.26 | 5.3 | 5.10 | 5.15 | 5.21 | 4.26 | 5.3 | 5.10 | 5.17 | 5.25 | 5.31 | 6.6 |
| 16:0al[b] | 2.2 | 1.6 | 1.6 | 0.8 | 1.2 | 2.2 | 2.0 | 1.3 | 1.2 | 1.8 | 1.6 | 1.7 | 1.7 |
| 16:0 | 15.9 | 18.0 | 19.9 | 22.0 | 20.5 | 20.7 | 16.9 | 19.2 | 20.0 | 19.0 | 21.6 | 21.2 | 20.7 |
| 16:1$_{\omega}$9 | — | — | 2.0 | 3.8 | 1.6 | 1.9 | — | — | 1.8 | 1.4 | 1.7 | 1.6 | 1.6 |
| 18:0al | 1.5 | 2.1 | 1.4 | 1.7 | 1.9 | 1.3 | 1.6 | 1.2 | 1.6 | 1.2 | 1.8 | 1.7 | 1.8 |
| 18:01 | 12.5 | 12.1 | 15.6 | 14.1 | 15.0 | 13.7 | 12.0 | 14.0 | 12.8 | 13.0 | 12.6 | 12.2 | 13.0 |
| 18:1$_{\omega}$9 | 13.0 | 17.7 | 20.5 | 24.4 | 21.8 | 20.2 | 13.9 | 18.0 | 19.6 | 20.7 | 20.0 | 19.3 | 19.1 |
| 18:2$_{\omega}$6 | 7.3 | 2.9 | 3.1 | 2.5 | 3.4 | 3.5 | 13.8 | 9.5 | 6.0 | 4.9 | 3.5 | 3.0 | 3.2 |
| 20:1$_{\omega}$9 | — | — | 0.6 | 0.5 | 0.6 | 0.5 | — | 0.6 | 0.4 | 0.5 | 0.4 | 0.3 | 0.3 |
| 20:2$_{\omega}$6,20:2$_{\omega}$9 | — | — | 0.6 | 0.5 | 0.5 | 0.3 | — | 0.5 | 0.4 | 0.5 | 0.5 | 0.4 | 0.4 |
| 20:3$_{\omega}$9 | — | 1.3 | 1.4 | 3.2 | 5.2 | 5.3 | — | 0.8 | 1.8 | 2.9 | 3.4 | 5.4 | 5.6 |
| 20:3$_{\omega}$6 | 2.9 | 2.5 | 1.9 | 0.5 | 0.7 | 0.6 | 2.5 | 2.9 | 2.0 | 2.1 | 1.0 | 0.9 | 1.4 |
| 22:0 | 1.0 | 1.9 | 1.2 | 1.2 | 0.6 | 0.2 | 1.1 | 1.6 | 1.5 | 1.1 | 0.9 | 0.5 | 0.6 |
| 20:4$_{\omega}$6 | 18.3 | 16.0 | 11.0 | 10.2 | 9.2 | 10.2 | 17.9 | 12.1 | 12.5 | 13.8 | 13.0 | 11.9 | 12.8 |
| 20:5$_{\omega}$3, 22:3$_{\omega}$9 | — | — | 0.9 | — | — | — | — | 1.2 | 1.0 | — | — | — | — |
| 24:0 | 8.9 | 10.0 | 7.2 | 6.2 | 6.9 | 9.0 | 7.8 | 7.8 | 7.6 | 6.4 | 7.1 | 9.1 | 7.9 |
| 22:4$_{\omega}$6 | 2.0 | 1.3 | — | — | — | — | 1.0 | — | — | — | — | — | — |
| 24:1$_{\omega}$9 | 6.9 | 7.9 | 6.2 | 5.2 | 5.7 | 5.8 | 8.3 | 5.8 | 5.6 | 6.5 | 7.0 | 7.6 | 6.7 |
| 22:5$_{\omega}$9 | 3.3 | 2.3 | 1.6 | 1.4 | 1.4 | 1.5 | 1.0 | 3.5 | 3.0 | 2.6 | 2.1 | 1.3 | 1.5 |
| 22:6$_{\omega}$3 | 3.6 | 4.5 | 2.7 | 2.1 | 3.8 | 2.9 | 2.5 | — | 1.5 | 1.1 | 1.8 | 1.9 | 1.7 |

[a] Patient and date.

[b] This shorthand abbreviation is explained in the text. al = aldehyde.

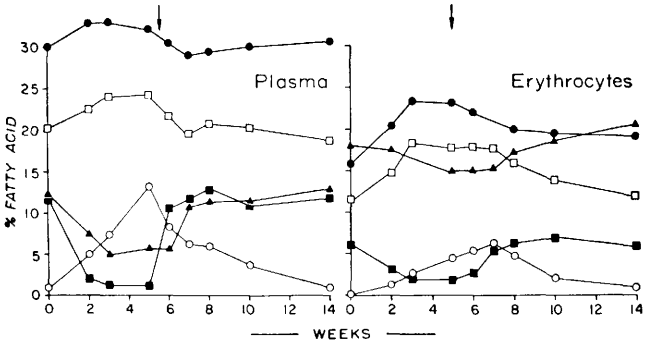


FIG. 1. Changes in the major phospholipid fatty acids of plasma and erythrocytes of a patient (E.H.) on intravenous hyperalimentation. The treatment started the first day after the first sample was analyzed (0 time) and continued for 5 weeks. At that time, it was discontinued and the patient was given orally regular food supplemented with safflower oil. (●) Palmitic acid; (□) oleic acid; (■) linoleic acid; (○) 5,8,11-eicosatrienoic acid, and (▲) arachidonic acid. Each point represents the mean of two determinations.

decreased to their control levels. Changes observed in the erythrocyte phospholipid fatty acids occurred more slowly although they followed a very similar pattern as the corresponding changes in plasma.

It is well-documented that changes in red-blood-cell phospholipids occur during circulation and that they are mediated by the interaction and exchange of blood cells with altered plasma lipoproteins. Qualitative and quantitative aspects of this exchange were discussed by several investigators including Sakagami *et al.* (21) and Soula *et al.* (22). Recently, Reed showed (23) that the actual amount of phospholipid turning over was small, with phosphatidylethanolamine and phosphatidylserine being nearly stable and with phosphatidylcholine and sphingomyelin having a turning over time of 5 days. It was postulated that the different rates and degree of exchangeability found for spingomyelin and phosphatidylcholine, in humans, depended not only on plasma concentration but on the orientation of the lipid molecule on the membrane and the nature of binding forces to membrane proteins. What effect the nature of the fatty-acid moiety has in this process has not been studied as yet. Several reports, however, have shown a very selective incorporation of fatty acids in rat red blood cells both *in vitro* (24, 25) and *in vivo* (26). The reaction is believed to be mediated by a transacylase similar to that reported in liver microsomes by Lands and Merkle (27) and Lands (28) and to account for a substantial proportion of lecithin turnover in rat erythrocytes. The process is known to occur in human red blood cells; however, the exact degree to which it occurs and its selectivity with respect to fatty-acid moieties is not known.

Regardless of the underlying mechanisms, the results in the present study show that the levels of polyunsaturated fatty acids (20:4ω6 and 20:3ω9) in red blood cells were more responsive to the state of essential fatty-acid deficiency when that was induced by intravenous hyperalimentation than by a long-term fat-free diet (18). Whether in the former case a more symmetrical exchange of phospholipid molecules takes place or a less selective transacylase operates remains to be seen.

*Summary.* Lipid analyses of erythrocytes from patients undergoing intravenous hyperalimentation therapy are presented herein. The amount of membrane cholesterol and phospholipid remained normal during the 4-month therapy and so did individual phospholipids. Phospholipid fatty acids, however, showed variations with a tendency toward increased percentages of palmitic and oleic acids and decreased percentages of linoleic and arachidonic acids. 5,8,11-Eicosatrienoic acid, characteristic of essential fatty-acid deficiency, increased markedly as the therapy progressed. Similar changes were observed in plasma fatty-acid phospholipids. The re-

sults suggested that during intravenous hyperalimentation, cell-membrane phospholipids became responsive to altered plasma-lipid concentrations. The exchange of phospholipids between red blood cells and lipoproteins in man is less selective in essential fatty-acid deficiency resulting from intravenous hyperalimentation than from other causes.

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1. Alfin-Slater, R. B., and Aftergood, L., Physiol. Rev. **48**, 758 (1968).

2. Holman, R. T., *in* "Progress in the Chemistry of Fats and Other Lipids" (R. T. Holman, ed.), Vol. IX, p. 279, Pergamon Press, New York (1968).

3. Wretlind, A., Nutr. Metab. Suppl. **14**, 1 (1972).

4. Collins, F. D., Sinclair, A. J., Royle, J. P., Coats, P. A., Maynard, A. T., and Leonard, R. F., Nutr. Metab. **13**, 150 (1971).

5. Wretlind, A., Nutr. Metabol. Suppl. **14**, 1 (1972).

6. Nelson, G. J., J. Lipid Res. **8**, 374 (1967).

7. Folch, J., Lees, M., and Sloane-Stanley, G. H., J. Biol. Chem. **226**, 497 (1957).

8. Zlatkis, A., Zak, B., and Boyle, A. J., J. Lab. Clin. Med. **41**, 486 (1953).

9. Bartlett, G. R., J. Biol. Chem. **234**, 446 (1959).

10. Sgoutas, D., Jones, R., and Leight, M., Intern. J. Biochem., **4**, 437 (1973).

11. Morris, L. J., J. Lipid Res. **7**, 717 (1966).

12. Hill, J. G., Kuksis, A., and Beveridge, J. M. R., J. Amer. Oil Chem. Soc. **41**, 393 (1964).

13. Hill, J. G., Kuksis, A., and Beveridge, J. M. R., J. Amer. Oil Chem. Soc. **42**, 137 (1965).

14. Neerhout, R. C., J. Lab. Clin. Med. **71**, 448 (1968).

15. Nye, W. H. R., and Marinetti, G. V., J. Clin. Invest. **44**, 1081 (1965).

16. Boon, J., Broekhuyse, R. M., Von Manster, P., and Schretlen, E., Clin. Chem. Acta **23**, 453 (1969).

17. Ways, P., Reed, C. F., and Hanahan, D. J., J. Clin. Invest. **42**, 1248 (1963).

18. Farquhar, J. W., and Ahrens, E. H., J. Clin. Invest. **42**, 675 (1963).

19. Greenburg, L. D., and Moon, H. D., Arch. Biochem. Biophys. **3**, 405 (1961).

20. Walker, B. L., and Kummerow, F. A., J. Nutr. **82**, 329 (1964).

21. Sakagami, T., Minari, O., and Orii, T., Biochem. Biophys. Acta **98**, 356 (1968).

22. Soula, G., Valdiguie, P., and Douste-Blazy, L., Bull. Soc. Chem. Biol. **49**, 1317 (1967).

23. Reed, C. F., J. Clin. Invest. **47**, 749 (1968).

24. Oliveira, M. M., and Vaughan, M., J. Lipid Res. **5**, 156 (1964).

25. Mulder, E., and van Deenen, L. L. M., Biochem. Biophys. Acta **70**, 106 (1963).

26. Mulder, E., de Grier, J., and van Deenen, L. L. M., Biochem. Biophys. Acta **70**, 94 (1963).

27. Lands, W. E. M., and Merkle, I., J. Biol. Chem. **238**, 898 (1963).

28. Lands, W. E. M., J. Biol. Chem. **235**, 2233 (1960).

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