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Selective incorporation of fatty acids into phospholipids of mature red cells

In order to verify a possible relationship between lipid composition and permeability properties of red-cell membranes[1,2] variations in the fatty acid composition of erythrocyte lipid were induced by dietary means. These experiments showed that significant quantitative alterations can brought about in the content of some fatty acid constituents of phospholipids from circulating erythrocytes, and that at a given regimen these variations reached their maximal extent within a period far shorter than the life span of the red cells[3] concerned. Further, it was shown that isolated erythrocytes readily incorporated [^{14}C]fatty acids into their phospholipids, while an ability of red cells to convert exogenous [^{32}P]lysolecithin into [^{32}P]lecithin was observed[3]. These results led us to the conclusion that certain fatty acid constituents of several major phospholipids present in the membrane of circulating erythrocytes are renewed by a transfer and incorporation of unesterified fatty acid from the plasma.

An incorporation of [^{14}C]palmitate into human blood cells was observed by MIRAS *et al.*[4], while in a recent abstract by OLIVEIRA AND VAUGHAN[5] it was reported that ghosts of human red cells can incorporate fatty acid in the presence of ATP and CoA, the uptake of linoleic acid being much greater than that of oleic or palmitic acid.

Further experiments guided by a dietary approach provided fresh evidence to support our former conclusion that incorporation *in vitro* of labeled fatty acids is related to the uptake of fatty acids in phospholipids from circulating red cells, by demonstrating an identical selectivity of both processes.

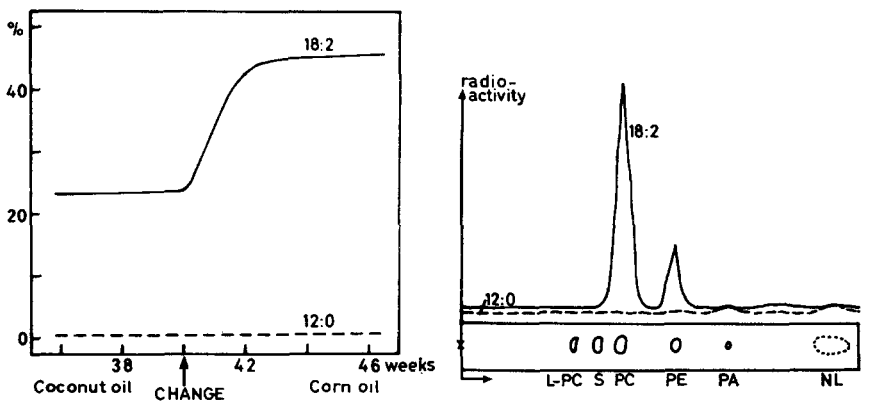

Fig. 1. Demonstration of the selective incorporation of fatty acids into phospholipids of red cells. A. Weight percentages of fatty acid methyl esters[1] in the phospholipid fraction of erythrocytes from rabbits fed on a diet high in coconut oil [containing 58% of lauric acid (abbreviated as 12:0)] and corn oil [60% linoleic acid (18:2)], respectively. B. Distribution of radioactivity on a paper chromatogram[2] of lipids extracted from rabbit erythrocytes, incubated in an equal volume of Ringer solution (0.2% glucose) containing [1-^{14}C]linoleic acid or uniformly labeled [^{14}C]linoleic acid (18:2) and [1-^{14}C]lauric acid (12:0), respectively, for 5 h at 37°. Abbreviations: L-PC, lysolecithin; S, sphingomyelin; PC, phosphatidyl choline or lecithin; PE, phosphatidyl ethanolamine; PA, phosphatidic acid; NL, neutral lipids.

Fig. 1A demonstrates the rapid increase of linoleate content in phospholipids of rabbit erythrocytes when their coconut-oil diet is replaced by a diet containing corn oil. Within 10 days the percentage of linoleic acid is doubled and attained the same level as animals nourished for one year on this regimen. When taking into account that the normal life span of rabbit erythrocytes is known to be 65 days, it is apparent that the observed switch in red-cell fatty acids is not due to maturation of red cells in the bone marrow. Actually after change of the diet the rise in linoleic acid in the red-cell lipids paralleled the increase in the plasma. The increase of linoleic acid in the red cell was localized mainly in the lecithin and phosphatidyl ethanolamine fraction and was accompanied by a loss mainly of oleic acid. In contrast to linoleic acid, ingestion of large amounts of lauric acid failed even after adminstration of coconut oil for 40 weeks to elevate the content of this fatty acid in the red-cell phospholipids, although the level of lauric acid in plasma was increased notably. The red cells did respond to a very limited extent to an increased intake of myristic acid. Hence, incorporation of fatty acids into red-cell phospholipids involves a mechanism discriminating between different types of fatty acids. This is true also with respect to incorporation *in vitro* of [^{14}C]fatty acids into phospholipids of erythrocytes washed free from plasma. As pointed out previously, incubation of red cells of normally fed rabbits, after removal of leucocytes by ultracentrifugation[6], in Krebs–Ringer solution (0.2 % glucose) without adding any cofactors, resulted in an incorporation of fatty acids. Fatty acids adhering to the cell surface were removed by treatment with isotonic saline containing albumin. After extraction of the lipids paper chromatograms were developed on silica-impregnated paper and the distribution of radioactivity was scanned. As reported before[3] linoleic acid was mainly incorporated into lecithin and into a less extent into phosphatidyl ethanolamine (Fig. 1B). A weak radioactivity was recovered in the glyceride fraction, while occasionally a labeling of phosphatidic acid was observed, this being significantly less than the radioactivity of the lecithin spot. A similar radioactivity distribution was observed after incubation periods varying from 30 min to 6 h. Incubation of erythrocytes of animal species low in lecithin content, *e.g.* bovine red cells[2], revealed a higher uptake of [^{14}C]linoleic acid into phosphatidyl ethanolamine. On the other hand, incubation of rabbit erythrocytes with [^{14}C]lauric acid failed to bring about any appreciable incorporation of this fatty acid into the red cells (Fig. 1B), while small amounts of [^{14}C]myristic acid were recovered. The agreement of these *in vitro* isotopic experiments with the results of the dietary experiments is evident, thus allowing the two experimental approaches to be correlated and permitting the conclusion that there is a selective mechanism of fatty acid incorporation into red-cell phospholipids.

Through the use of snake-venom phospholipase A (EC 3.1.1.4), known to liberate fatty acids from the β-position only[7,8], we found the incorporated [^{14}C]linoleic acid to be located mainly in the β-ester position of the lecithin. Inasmuch as HANAHAN *et al.*[8] demonstrated that in lecithin of human erythrocytes saturated and unsaturated fatty acids are located mainly at the γ- and β-position, respectively, it might be presumed that the observed location of the incorporated [^{14}C]linoleic acid had to be attributed to the positional specificity of the acyl transferase as reported by LANDS AND MERKL[9]. However, our observation on the difference of the rate of incorporation between lauric acid and linoleic acid appeared not to be merely a difference between saturated and unsaturated fatty acid. Palmitic acid was more readily incorporated than lauric acid,

although at a lower rate than linoleic acid, this being in agreement with the observations of OLIVEIRA AND VAUGHAN[5]. This difference between palmitic acid and lauric acid is the more surprising since it has been demonstrated that lauric acid can replace unsaturated fatty acid constituents in monomolecular layers of synthetic lecithins with little change in the interfacial properties[10].

These observations point the way to a final elucidation of the pathway(s) involved in fatty acid incorporation into red-cell phospholipids. It must be determined whether the reported ability of red cells to convert lysolecithin into lecithin[3,5] holds true also for the circulating red cell. A synthesis *de novo* of phospholipids must also be envisaged. Available information on the incorporation of [^{14}C]fatty acids and the low uptake of radiophosphate[11,12] does not support the presence of a net synthesis of phospholipids in the mature red cell, but further experimental evidence is required. Another problem represents the removal of fatty acid constituents from the red-cell lipids. As noted before, particularly oleic acid, present in β-position of red-cell phosphoglycerides, is being interchanged with linoleic acid. Therefore, it is attractive to speculate on a functioning of a β-specific transacylase in the red-cell membrane. Actually we observed such an enzyme to be attached to the surface of the red cell, but it remains to be established whether this enzyme participates in the processes discussed.

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