LIPID METABOLISM OF MAMMALIAN ERYTHROCYTES WITH SPECIAL REFERENCE TO CELLULAR AGING

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ABSTRACT

Aspects of the lipid metabolism of mammalian erythrocytes, with special reference to changes in aging cells, have been investigated.

Bovine erythrocytes and leukocytes have been incubated with labelled palmitic acid, and the incorporation of the fatty acid into each cell type has been followed. A high level of incorporation was observed with leukocytes, mainly into the phospholipids and triglycerides. Incorporated palmitate took part in chain-lengthening processes and some $^{14}CO_2$ was produced during the incubations. Incorporation into the lipids of erythrocytes was very much lower than that observed for leukocytes and low leukocyte concentrations in red cell preparations accounted for a significant proportion of [1-^{14}C]palmitate uptake into the cell lipid. The importance of accounting for the metabolic activities of residual leukocytes has been stressed. After allowing for leukocyte contributions, a significant incorporation of palmitate into erythrocyte phospholipids, in particular phosphatidyl choline and phosphatidyl ethanolamine, was demonstrated. However, no significant uptake into the small quantities of triglyceride or cholesterol esters present in the erythrocytes could be detected.

Experiments have been carried out to examine variations in lipid content with cell age, in bovine erythrocytes fractionated by serial osmotic hemolysis. Only slight differences in cellular phospholipid or cholesterol content were found, and cholesterol:phospholipid ratios were constant in all fractions. No marked variation in cholesterol ester, triglyceride, or free fatty acid concentration with cell age could be detected.

Human red cells have been fractionated according to age by ultracentrifugation over discontinuous albumin density gradients. The efficiency of such age separation was examined by following the radioactivity distribution in the gradient when rat cells were fractionated at intervals after administration of reticulocytes labelled with [^{14}C]glycine. Considerable localisation of cells of particular ages in specific density bands was observed. Variations in lipid composition and in fatty

acid uptake into the cells have been investigated. A small decrease in lipid content with cell age was detected, the decrease being most marked between the youngest and all the other fractions. It is suggested that all the changes in lipid content which occur in aging red cells could take place during the transition from reticulocyte to erythrocyte.

Incorporations of labelled linoleate into intact red cells, and linoleate and palmitate into ghosts enriched with ATP and CoA have been examined. The major cell lipids which took up the acids were phosphatidyl choline, phosphatidyl ethanolamine, components tentatively identified as phosphatidic acid and diglyceride, and an unidentified non-polar lipid. A wide range of behaviour has been observed for different normal cell populations, both in total uptake, and in the distribution of the incorporated acid. In most cases uptake was predominantly into phosphatidyl choline, but in others uptakes into phosphatidyl choline and phosphatidyl ethanolamine were comparable. The observed range of behaviour can be explained as arising from differences in concentrations of the substrates required for fatty acid incorporation in the plasma in which the cells or ghosts were incubated.

Fatty acid uptakes into red cells and ghosts were also studied as a function of cell age. A wide diversity of behaviour for different blood samples was apparent. In some cases, uptake into all components was essentially independent of cell age, but in others, uptakes into specific components showed definite trends with age. Most noteworthy were a marked increase with age in uptake into phosphatidyl ethanolamine, with essentially constant uptake into phosphatidyl choline, and a decrease in uptake into phosphatidyl choline with uptake into phosphatidyl ethanolamine remaining constant. To account for the diversity of behaviour, it is suggested that changes in either enzyme availability or conformation, affecting cellular enzyme activity, occur as the cells age, and that only for certain plasma concentrations of the substrates required for fatty acid uptake, are these changes in enzyme activity evident.

In a single study, bovine erythrocytes have been labelled *in vivo* with [^{14}C]acetate, and levels of activity in the cell lipids followed. A decline in free cholesterol activity, arising from rapid equilibration with plasma cholesterol, has been demonstrated. A fall in activity that could be interpreted in terms of red cells being able to exchange some but not all of their phospholipids with plasma counterparts has also been found.

Roles for triglycerides and cholesterol esters in the erythrocyte, and properties of the erythrocyte phospholipid transacylation mechanism have been discussed. The possible importance of changes in lipid constitution and lipid metabolism in red cell aging has been considered.

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