Phosphatide patterns of normal, spherocytic and elliptocytic red blood cells

In the course of an investigation[1,2] into the lipid composition of red cells it was considered of interest to extend our studies to pathological cases *e.g.* elliptocytosis and spherocytosis. While this work was in progress, ALLISON *et al.*[3,4] reported that the percentage of phosphatidyl ethanolamine was considerably decreased in spherocytes when compared with normal red-blood cells. Our results do not agree with this observation.

Normal and pathological red cells were haemolysed according to the CO_2 method[5] and lipids were extracted from the collected ghosts by treatment with ethanol–ether (3:1, v/v). Determinations of the main types of phosphatides were carried out by quantitative chromatography on silica-impregnated paper, as outlined by MARINETTI *et al.*[6]. Details of the various methods involved were already described in a previous paper[7].

TABLE I

PROPORTIONS OF MAJOR PHOSPHATIDES IN GHOSTS OF NORMAL HUMAN RED CELLS, SPHEROCYTES AND ELLIPTOCYTES

Data are given as percentages of total phosphatides. The standard errors are placed between parentheses.

Red blood cells	*Number of subjects*	*Lysolecithin (%)*	*Sphingolyelin + lysophosphatidyl ethanolamine (%)*	*Lecithin (%)*	*Phosphatidyl ethanolamine (%)*
Normal	7	2 (1.5)	32 (1.8)	36 (1.0)	30 (1.5)
Spherocytic	7	2 (0.5)	32.5 (1.4)	36 (1.5)	29.5 (1.5)
Elliptocytic	3	2 (0.6)	32 (1.7)	37 (2.4)	29 (2.2)

The results summarized in Table I demonstrate that the proportions of the major classes of phosphatides from red-cell ghosts of seven subjects (two males and five females) with typical hereditary spherocytosis did not differ significantly from the values normally obtained. The same was found to be true in the case of hereditary elliptocytosis. In spherocytosis no differences were found in phosphatide pattern between patients with or without spleen, nor were any differences found in the phosphatide compositions of two subjects before and after splenectomy.

It is clear that a decrease of the percentage of phosphatidyl ethanolamine in ghosts of spherocytic cells, when compared with normal cells, was not encountered in the present study. ALLISON *et al.*[3,4] reported that in two cases of hereditary spherocytosis investigated the observed appreciable decrease of phosphatidyl ethanolamine was exactly compensated by an increase of a component probably identical with a lysophosphatidyl ethanolamine. These authors therefore assumed that in hereditary spherocytosis a partial block may exist in a bio-synthetical conversion of lysophosphatidyl ethanolamine into phosphatidyl ethanolamine. In addition it was observed that the lysophosphatidyl ethanolamine compound, just like lysolecithins, caused normal red cells to become spherical. Therefore, ALLISON *et al.*[3,4] concluded that the most likely primary defect in hereditary spherocytosis is in the final stage of the synthesis of phosphatidyl ethanolamine. Recently, LANDS[8] reported that a conversion of

lysolecithin into lecithin may actually play a part in phosphatide metabolism of rat-liver microsomes.

The discrepancy between the results of ALLISON *et al.*[3,4] and those brought forward in the present paper may have been caused by differences in the materials studied. On the other hand, we have already shown that phosphatidyl ethanolamine of human red cells, which compound was found to give a positive reaction for plasmalogens[9] (compare also DAWSON *et al.*[10]), is a rather unstable component[7]. Upon storage of dry lipid samples from normal, spherocytic and elliptocytic red cells *in vacuo* over P_2O_5 at 2° in the dark, the greater part of phosphatidyl ethanolamine appeared to decompose, thereby yielding a component behaving paper-chromatographically similar to lysophosphatidyl ethanolamines obtained by snake-venom degradation of synthetic mixed-acid phosphatidyl ethanolamines[11]. In addition, dissolving the lipid samples in chloroform containing phosgene, as well as treatment with acetic acid for the release of aldehydes from plasmalogens according to GRAY AND MACFARLANE[12], brought about a similar degradation of this red-cell phosphatide. In general, the resulting lipid samples from both normal and pathological cells revealed after such treatments percentages of phosphatidyl ethanolamine which were even lower than the values reported by ALLISON *et al.*[3,4] to exist in hereditary spherocytosis. It is worth noting that HANAHAN *et al.*[13] observed that, when human blood was collected in heparin, phosphatidyl ethanolamine of red cells tended to form lyso derivatives. The chemical structure of this compound, as well as the lipid compostion of pathological red cells are being further investigated.

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Received May 6th, 1961