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RED CELL SURFACE STRUCTURE

STABILIZATION BY CHOLESTEROL SULFATE AS EVIDENCED BY SCANNING ELECTRON MICROSCOPY

GILLES BLEAU, GASTON LALUMIÈRE, ALCIDE CHAPDELAINE and KENNETH D. ROBERTS

Departments of Biochemistry and of Medicine, University of Montreal and Maisonneuve-Rosemont Hospital, 5415 Blvd. de L'Assomption, Montreal, P. Quebec, H1T 2M4 (Canada)

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SUMMARY

Scanning electron microscopic studies demonstrate that the normal biconcave shape of the human erythrocyte is maintained in hypotonic saline when physiological levels (10^{-5} M) of cholesterol sulfate are added to the solutions. Cholesterol sulfate is a naturally occurring sterol conjugate in plasma and erythrocyte membranes and we propose that it may belong to that class of amphipathic molecules responsible for the maintenance of structure of the erythrocyte by interaction with membrane components.

Cholesterol sulfate is a component of the human erythrocyte membrane and plasma [1–3]. This sterol conjugate significantly decreases the osmotic fragility of erythrocytes exposed to hypotonic saline solutions [3]. This effect involves some degree of specificity since the sterol side-chain and the sulfate moiety are both essential for activity [3].

The following experiments describe the effect of cholosterol sulfate on the membrane of the human erythrocyte. Blood from a normal male was collected in EDTA. Following separation of the red cells by centrifugation, the cells were washed twice with 0.9 % NaCl-containing 0.1 M phosphate buffer (pH 7.2). The washed cells were reconstituted to the original volume of whole blood with physiologic saline. 50 μl of this cell preparation was added to: (1) 5 ml of 0.425 % NaCl-containing 0.1 M phosphate buffer (pH 7.2) and (2) the same solution containing 10^{-5} M cholesterol sulfate. In order to ensure a complete solution of the sterol conjugate in the aqueous medium, 50 μl of ethanol was added to the tube containing the conjugate prior to the addition of the saline-buffer medium. The same volume of ethanol was added to the control tube. After standing at room temperature for 1h, aliquots were removed for scanning electron microscopy as well as for phase contrast microscopy.

For scanning electron microscopy, the erythrocytes were fixed in 3 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and washed twice with phosphate buffer. Following concentration of the red cells on a Millipore filter (0.25 μm pore size)

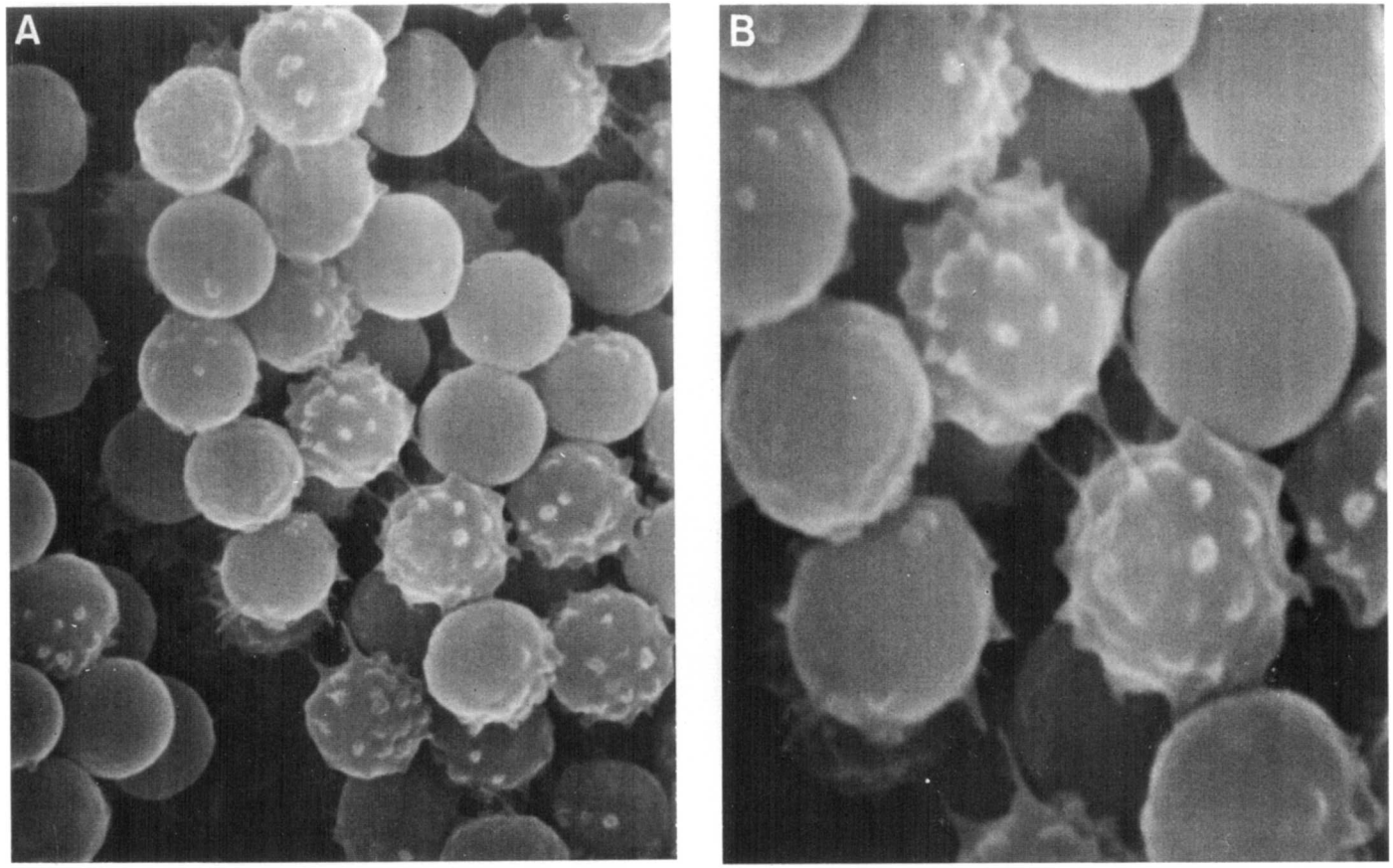

Fig. 1a. Scanning electron micrograph of human erythrocytes in hypotonic saline solution. × 10 000. b. Scanning electron micrograph of human erythrocytes in hypotonic saline solution. × 20 000.

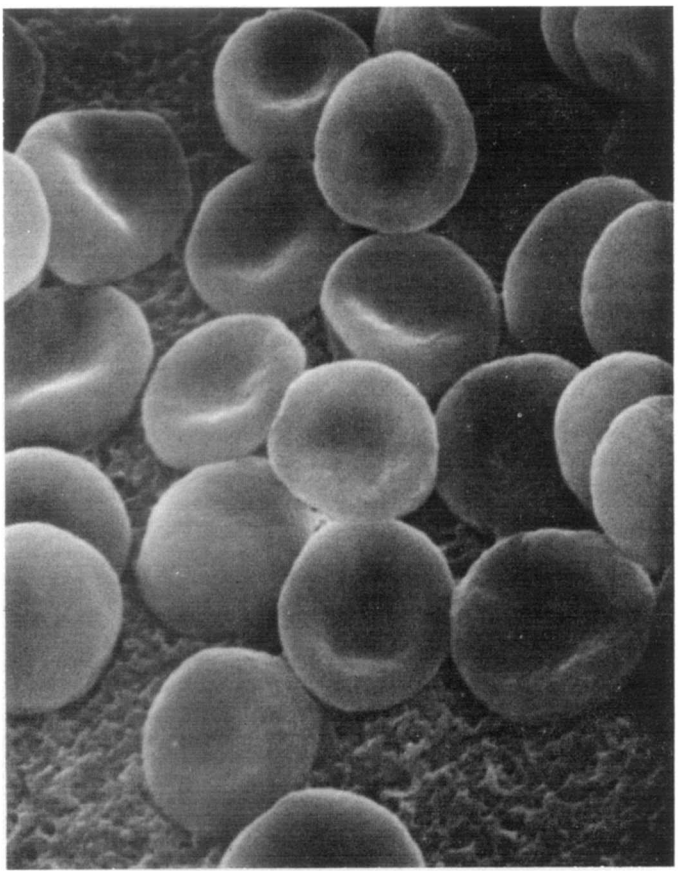

Fig. 2. Scanning electron micrograph of human erythrocytes in hypotonic saline solution in presence of 10^{-5} M cholesterol sulfate. ×10 000.

the specimen was mounted on a specimen stub and air dried. The erythrocytes were then vacuum-coated with carbon and gold and examined with a scanning electron microscope (RMR-900).

As shown in Fig. 2, the normal smooth biconcave shape of the red cell is retained in the presence of cholesterol sulfate. In contrast, the absence of cholesterol sulfate causes the erythrocytes to assume a spherical shape, to extend spicules and also appear to form intercellular bridges indicating a "sticky" nature of the cell surfaces (Fig. 1, a and b).

This finding was corroborated, although in much less detail, by phase contrast microscopy of the cells in the saline solutions. This would eliminate the possibility of artifacts arising from the technique of electron microscopy.

It is recognized that the stabilizing effect of cholesterol sulfate on the erythrocyte membrane has been observed, up to the present time, only under non-physiological conditions i.e. in hypotonic media. Whether the sterol conjugate plays a role in the maintenance of membrane structure in vivo remains to be established. Nevertheless its presence in human plasma and the red cell membrane, its uptake by the

dog erythrocyte membrane in vivo [4] and by the rat erythrocyte [5] as well as the specificity of this uptake certainly merit further investigation. It was of interest in this study to observe that at the osmolar salt concentration used, the erythrocytes, in the absence of cholesterol sulfate, are not unlike those obtained from patients with membrane fragility disorders such as hereditary spherocytosis. An attempt to correlate the degree of red cell fragility in pathological conditions with the circulating levels and/or the uptake of cholesterol sulfate by the erythrocyte is currently in progress.

Finally, an important role of monovalent cations in the structural stability of the erythrocyte membrane has been suggested by Reynolds [6]. This author reports that zwitterionic lipids (such as lecithins and phosphoethanolamines) are much less likely to interact with inorganic cations than anionic lipids. "Thus, minor lipid constituents could well be responsible for the structural and functional characteristics of biological membranes". One minor anionic lipid constituent to be considered is cholesterol sulfate.

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