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THE RELEASE OF MEMBRANE COMPONENTS PRIOR TO HAEMOLYSIS DURING EXTRACTION OF INTACT ERYTHROCYTES WITH BILE SALTS

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Summary

Glycocholate removed significant amounts of acetylcholinesterase and membrane phospholipid from human erythrocytes prior to cell lysis. The phospholipids were relatively enriched in phosphatidylcholine. These results may represent selective attack on the outer leaflet of the plasma membrane and also may provide a model for some aspects of hepatic bile formation.

In a recent study [1] we reported that the trihydroxy bile salt, cholate, and its conjugates produced a unique pattern of solubilization of membrane proteins from erythrocyte ghosts and left behind a residue which still resembled a membrane by electron microscopy. We report here the ability of these detergents to remove components from the erythrocyte membrane prior to the occurrence of significant cell lysis.

Human erythrocytes were extensively washed and resuspended in 0.154 M NaCl 1.5 mM HEPES buffer, pH 7.4, to a concentration of approximately 2 μmol phospholipid per ml. One volume of this suspension was added to 3 volumes of 0.14 M NaCl, 15 mM HEPES, pH 7.4 containing different amounts of detergent to give the final concentrations shown. The mixtures were incubated at 37°C for 10 min and then centrifuged in a microcentrifuge at 14 000 × g for 2 min, and the supernatant carefully removed from the packed cells. In some experiments the supernatants were further centrifuged at 150 000 × g for 60 min to yield high speed supernatants and pellets.

The extent of haemolysis was assayed by comparing the absorbance at 525 nm of 20-fold dilutions of the supernatants with a 20-fold dilution (with water) of an uncentrifuged control. Lipid extracts [2] of supernatants and pellets were assayed for phospholipid phosphorus by the methods of King [3]

or Bartlett [4], and their phospholipid profiles were obtained by thin-layer chromatography [5]. Acetylcholinesterase activities were assayed according to Ellman [6], and corrections were made for detergent inhibition by comparison with appropriate uncentrifuged detergent-containing and detergent-free controls.

Cholate and deoxycholate were obtained from Sigma Chemical Co., London, glycodeoxycholate, glycocholate and taurocholate from Calibiochem Ltd., Hereford, U.K., Triton X-100 from Rohm and Hass Ltd., Croydon, U.K., and Tween 20 from Koch-Light Laboratories, Colnbrook, U.K.

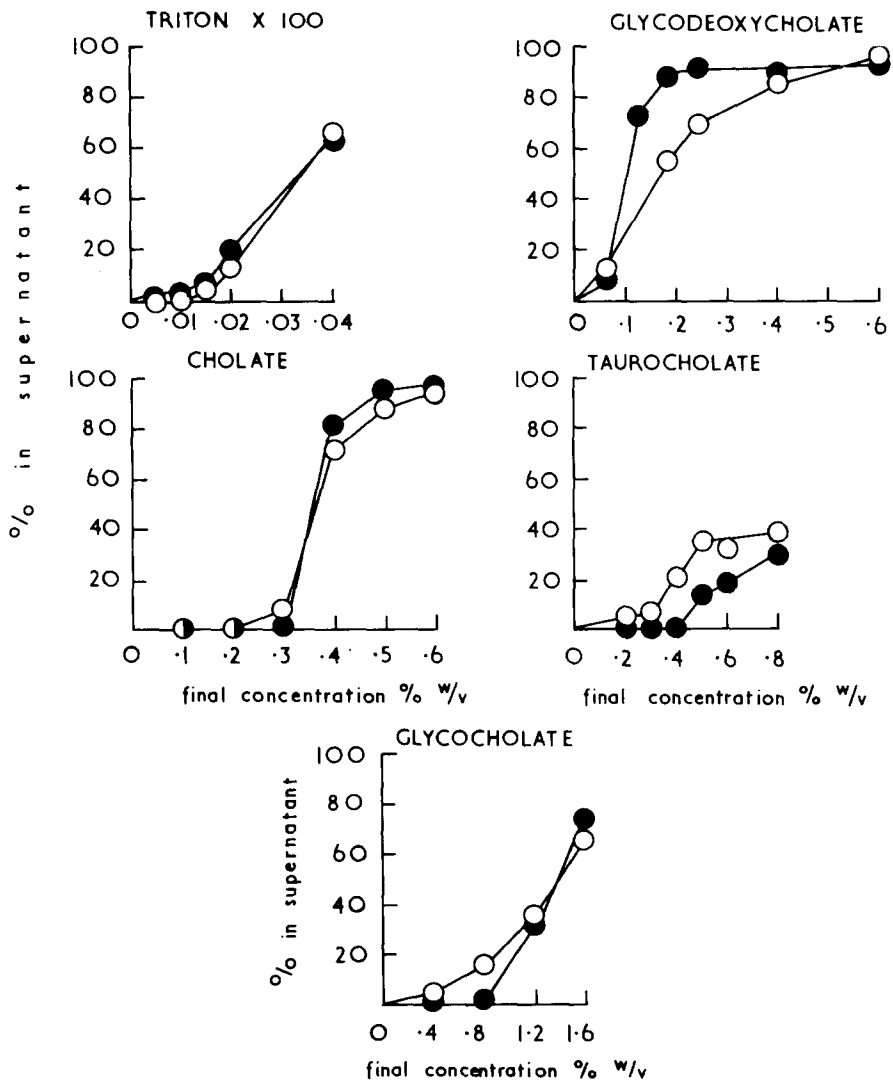

Fig.1. Release of materials from intact erythrocytes. Erythrocytes were incubated at 37°C for 10 min at the final detergent levels shown. Supernatants were obtained after centrifugation at 14 000 X *g* for 2 min. Each experiment shown is typical of several, though the concentration at which lysis occurred showed slight variance from preparation to preparation of erythrocytes. •,haemoglobin; ○, phospholipid.

The release of phospholipid into the supernatant, in relation to the amount of haemolysis brought about by several detergents, is shown in Fig. 1. In the case of Triton X-100 and Tween 20, at all levels, and deoxycholate, glycodeoxycholate and cholate at moderate levels, haemolysis equalled or preceeded the loss of phospholipid into the supernatant.

Low levels of deoxycholate and glycodeoxycholate liberated a small amount of phospholipid in excess of the amount of haemolysis and this phenomenon was more marked with cholate, where about 10% (range 3—15%) was liberated prior to lysis. Taurocholate and glycocholate extracted more phospholipid than cholate prior to the region in which significant lysis occurred, which was usually in the region of 15% phospholipid release.

Cholate and taurocholate, however, gave inconsistent results. The release of phospholipid prior to haemolysis was observed in only 6 of 9 and 5 of 8 experiments, respectively. The reasons for this variability are not yet clear; there are some indications that it is correlated with the age and concentrations of the erythrocytes used and, in the case of taurocholate, with possible impurities in one of the bile salt samples.

Further studies were, therefore, restricted to glycocholate. This gave a variety of shape changes by phase contrast and electron microscopy in the pre-lytic stage. Most common were cup-shaped forms, but there was no consistant evidence of internal microvesicles or microprojections. The phospholipid released did not represent the overall phospholipid composition of the original ghosts; it was relatively enriched in phosphatidylcholine and relatively depleted in phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol. The sphingomyelin content was approximately the same as in the original ghosts (Table I).

TABLE I

COMPARISON OF THE PHOSPHOLIPIDS OF A GLYCOCHOLATE EXTRACT (0.6%) OF RED BLOOD CELLS WITH CONTROL CELLS

Intact erythrocytes were incubated at 37°C for 10 min at a concentration of 0.6% glycocholate. The erythrocytes were then sedimented at 14 000 × g_{av} for 2 min and the supernatant further centrifuged at 150 000 × g_{av}. Lipid extraction [2] and phospholipid chromatography [5] were carried out against authentic phospholipid standards. Spots were quantified by phosphorus determination. The results represent the means of 4 experiments (erythrocytes) and 11 experiments (glycocholate extract).

	Erythrocyte lipids	Glycocholate ext.
Solvent front	3	3
Phosphatidylethanolamine	25	12
Phosphatidylserine and phosphatidylinositol	13	3
Phosphatidylcholine	30	54
Sphingomyelin	25	24
Origin	3	3

Fig. 2 shows the release of acetylcholinesterase activity into the supernatant in comparison to that of phospholipid and haemoglobin. Approximately 30% of the total activity of the cells was removed prior to haemolysis.

The loss of phospholipid from whole red cells without lysis is reminiscent of the loss of phospholipid resulting from the action of certain phospholipases. In these cases it is the outer leaflet of the plasma membrane that has been suggested to have been affected [7].

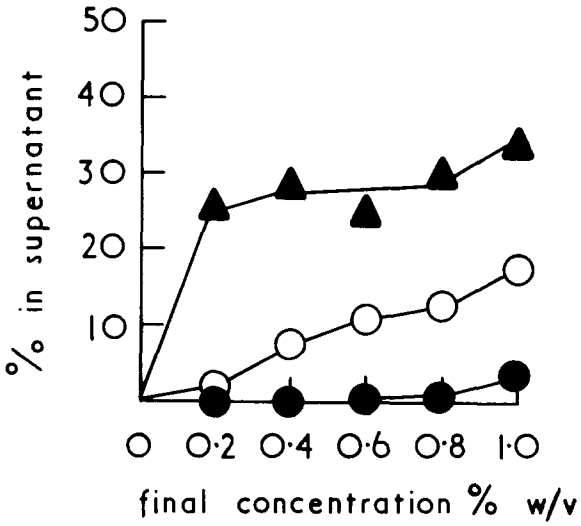

Fig.2. Glycocholate-induced release of acetylcholinesterase from intact erythrocytes. Erythrocytes were incubated at 37°C for 10 min at the final detergent levels shown. Supernatants were obtained after centrifugation at 14 000 × *g* for 2 min. Points represent means of 10 experiments. ▲, acetylcholinesterase; ○, phospholipid; ●, haemoglobin.

The phospholipid profile of the outer leaflet of the human erythrocyte membrane is thought to be largely composed of phosphatidylcholine, sphingomyelin and only small amounts of phosphatidylethanolamine [7]. The composition of the material extracted with glycocholate more closely resembles this composition thanthat of the intact membrane [7] and it is possible glycocholate has removed components from the outer leaflet of the membrane without causing sufficient damage to the overall membrane structure to result in lysis. The release of acetylcholinesterase by glycocholate prior to haemolysis indicates that some proteins also may be released from the membrane without grossly disrupting the membrane structure. It is well documented that this enzyme has on outward facing orientation [8,9] although the nature of its association with other membrane components is not yet fully understood.

It is possible that this selective activity of some bile salts at the surface of the red cell may be a model for the activity of these detergents at the hepatocyte cell surface during, or more probably after, bile salt liberation into the liver bile canaliculus.

In bile, the bile salts are accompanied by quantities of phospholipid, largely phosphatidylcholine and sphingomyelin (see ref. 10), by cholesterol and by glycoprotein enzymes e.g. 5′-nucleotidase, alkaline phosphatase, L-leucyl-β-naphthylamidase and alkaline phosphodiesterase I [11]. These enzyme activities are normally used to characterize plasma membranes and have been shown to be located on the outer face of the plasma membrane i.e. "ectoenzymes". (see ref. 11). They are not accompanied, in bile, by enzymes indicative of excessive cell destruction [12]. The ability therefore of some bile salts to remove material from intact cells, from the outside, without resulting in cell lysis may indicate a way that the plasma membrane

of liver cells may make a contribution to the composition of bile without resulting in the complete death of the cell.

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