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Plasma-Membrane Components Can be Removed from Isolated Lymphocytes by the Bile Salts Glycocholate and Taurocholate without Cell Lysis

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Glycocholate and taurocholate removed from isolated pig lymphocytes a proportion of the cells' complement of 5′-nucleotidase, alkaline phosphatase and alkaline phosphodiesterase I before cell lysis. This may indicate a loss of externally orientated plasma-membrane components.

The cholate group of detergents (cholate, taurocholate and glycocholate) appear to be relatively mild in their effects on biological membranes and display particular discrimination in their release of proteins from the membranes of erythrocyte 'ghosts'. They leave behind a lipid-depleted membrane-like residue, which still gives a trilaminar image by transmission electron microscopy and is largely composed of spanning intrinsic proteins (Coleman *et al.*, 1976*a*).

Further, when glycocholate is presented to intact erythrocytes it can cause the release of a proportion of the membrane complement of acetylcholinesterase and phospholipid before the onset of lysis (Coleman & Holdsworth, 1976). Acetylcholinesterase is an externally orientated erythrocyte membrane protein (Steck, 1974) and the phospholipids released also represent predominantly externally orientated types (Renooij *et al.*, 1976).

Mammalian bile contains high concentrations of bile salts, of which the conjugated cholate derivatives form a large part; the bile salts are present as mixed micelles with phospholipid and cholesterol (Heaton, 1972). Bile also contains significant amounts of the plasma-membrane enzymes 5′-nucleotidase, alkaline phosphatase, alkaline phosphodiesterase I and L-β-naphthylamidase; it therefore shows an apparent correlation with the enzymology of the liver plasma membrane (Holdsworth & Coleman, 1975*a*). These enzymes are glycoproteins and are externally orientated on the plasma membrane (see Holdsworth & Coleman, 1975*b*). The absence from bile, however, of significant amounts of intracellular enzymes suggests that the plasma-membrane enzymes may be released by a process that does not involve cell breakage (Holdsworth & Coleman, 1975*a*).

To examine further the abilities of some biliary detergents to liberate plasma-membrane components from cells without causing lysis, we have examined their effects on isolated pig lymphocytes. Like liver cells and bile these cells possess 5′-nucleotidase, alkaline phosphatase and alkaline phosphodiesterase I activities. The 5′-nucleotidase has been shown to be an externally orientated plasma-membrane enzyme (Misra *et al.*, 1974) and, by analogy with other cells (e.g. see DePierre & Karnovsky, 1973), the other two enzymes might also be expected to be externally orientated plasma-membrane enzymes. Lactate dehydrogenase was used as an appropriate enzyme to indicate cell lysis. As a comparison and control we have also studied the effects of Triton X-100.

Experimental

Pig mesenteric lymph nodes were obtained fresh from a local slaughterhouse and isolated lymphocytes were prepared in iso-osmotic Krebs–Ringer/Hepes buffer, pH 7.1, by the method of Allan & Crumpton (1970).

Glycocholate and taurocholate were from Calbiochem, Hereford, U.K., and Triton X-100 was from Rohm and Haas, Croydon, U.K.

The isolated cells were suspended in the above buffer and incubated for 10 min at 37°C with the final concentrations of detergent shown, at a concentration of approx. 3×10^{8} cells/ml. The mixtures were then centrifuged at 14000g for 2 min in a microcentrifuge and the resulting supernatants were carefully removed. In the experiments were included detergent-free incubations with and without centrifugation to provide background and 100% values respectively for the various parameters under investigation.

The following enzyme assays were carried out on supernatants or cell suspensions: 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5; Michell & Hawthorne, 1965), alkaline phosphodiesterase I (EC 3.1.4.1; Brightwell & Tappell, 1968); alkaline phosphatase (EC 3.1.3.1; Connock *et al.*, 1971); lactate dehydrogenase (L-lactate–NAD^{+} oxidoreductase, EC 1.1.1.27; Wroblewski, 1955); arylsulphatase (EC 3.1.6.1; Shephard &

Hübscher, 1969); succinate dehydrogenase (EC 1.3.99.1; Pennington, 1961).

Sonication was performed at 0°C for 20s with an MSE sonicator (1cm probe) operating at 60W and 20kHz at maximum power.

Results

In these experiments, cell lysis was marked by the appearance, in the supernatant after incubation, of the intracellular enzyme lactate dehydrogenase (Figs. 1 and 2; solid lines). Other intracellular enzymes, arylsulphatase (endoplasmic reticulum) and succinate dehydrogenase (mitochondria), accompanied or followed lactate dehydrogenase in the lysis sequence (Fig. 1, inset).

When lymphocytes were incubated with increasing amounts of glycocholate or taurocholate, a considerable proportion of the cellular complement of 5′-nucleotidase, alkaline phosphatase and alkaline phosphodiesterase I appeared in the supernatant before lysis (Figs. 1 and 2*a*). Taurocholate was somewhat more effective than glycocholate.

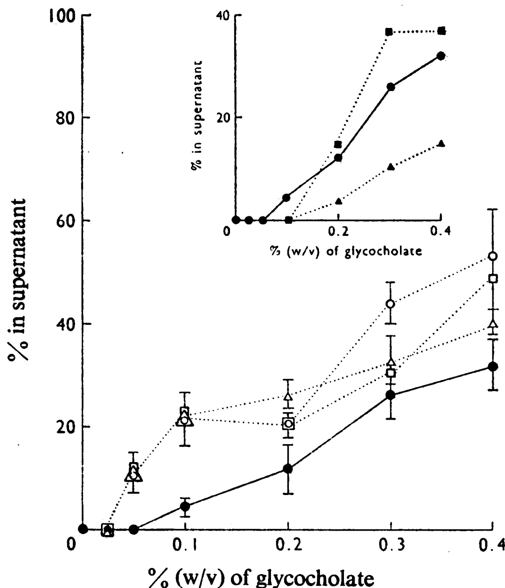

Fig. 1. *Release of enzymes from isolated lymphocytes with increasing concentration of glycocholate*

For experimental details see the text. The main graph shows the release of putative plasma-membrane enzymes in relation to lactate dehydrogenase. The points represent the means±S.E. of six experiments. The inset shows the release of intracellular enzymes (one experiment). ●, Lactate dehydrogenase; □, alkaline phosphatase; ○, alkaline phosphodiesterase; △, 5′-nucleotidase; ■, arylsulphatase; ▲, succinate dehydrogenase.

For Triton X-100, the onset of the release of these plasma-membrane enzymes was much closer to the point of lysis, and the amounts liberated were small. Further amounts of these enzymes were then released, but at a lower rate than the intracellular enzyme, lactate dehydrogenase (Fig. 2*b*).

Comparison of the activities of 5′-nucleotidase, alkaline phosphodiesterase I and alkaline phosphatase, in intact and sonicated preparations, showed correspondence to within 10%, suggesting that these enzymes were probably not latent in the lymphocyte preparation.

Discussion

Before the release of intracellular proteins, a proportion of the cellular complement of 5′-nucleotidase, alkaline phosphatase and alkaline phosphodiesterase could be removed from intact lymphocytes by taurocholate and glycocholate. It is possible that such release before lysis is an indication of the outward orientation of these enzymes in the plasma membrane, since these detergents have been shown to be able to remove outward-facing components from the human erythrocyte membrane (Coleman & Holdsworth, 1976). Such components would probably be located in the outer leaflet of the lipid bilayer since, even at high concentrations, the cholate group of detergents failed to release spanning proteins from erythrocyte membranes (Coleman *et al.*, 1976*a*).

The outward-facing orientation for 5′-nucleotidase, alkaline phosphatase and alkaline phosphodiesterase is supported by the non-latent nature of these enzymes in intact lymphocytes, as shown in the present study and by Misra *et al.* (1974), and by analogy with the disposition of these enzymes in other cells (see the introduction).

Although the conjugated trihydroxy bile salts appeared to be able to remove significant amounts of plasma-membrane enzymes without causing lysis, Triton X-100 appeared to have much less ability to achieve this and produced lysis more readily. In other experiments we have shown that Triton X-100 (and deoxycholate) readily cause lysis of intact human erythrocytes without the previous release of appreciable amounts of membrane components (Coleman & Holdsworth, 1976). In experiments with erythrocyte 'ghost' preparations we have also shown that Triton X-100 (and deoxycholate) are much more vigorous in their action and give rise to greater disorganization of the membrane than do taurocholate, glycocholate and cholate (Coleman *et al.*, 1976*a*).

Our studies indicate the potential value of glycocholate and taurocholate in probing the composition and structure of the outer leaflet of the plasma membrane of the cell. Also, the action of these mild detergents on the outside face of the lymphocyte plasma membrane may be analogous to the action of

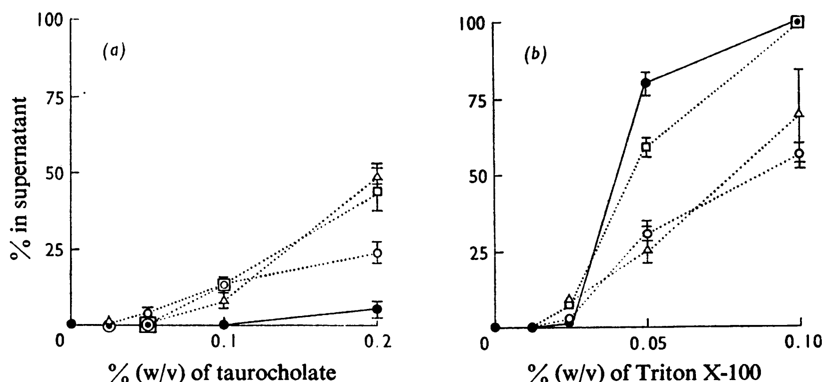

Fig. 2. *Release of enzymes from isolated lymphocytes with increasing concentrations of taurocholate and Triton X-100*

For experimental details see the text. (*a*) Taurocholate; (*b*) Triton X-100. The results represent the means±S.E. of four experiments for (*a*) and two experiments for (*b*). The symbols are as for Fig. 1.

bile salts on the outside face of the plasma membrane of the hepatocyte and therefore provide support for a model (Holdsworth & Coleman, 1975*b*; Coleman & Holdsworth, 1976; Coleman *et al.*, 1976*b*; Evans *et al.*, 1976) for the behaviour of bile salts during, or more probably after, their release from the liver cell. The essential features of this model are that the newly externalized bile salts (with a composition containing a high proportion of the less severely damaging types) are pumped out of the cell by an active-transport process. These accumulate in the bile canaliculus, where they form micelles, which then withdraw material from the outer leaflet of the plasma membrane of the liver cell, but without causing lysis. This model is consistent with the known composition of bile, the phospholipids of which are predominantly phosphatidylcholine, and with the presence of the externally facing plasma-membrane enzymes 5′-nucleotidase, alkaline phosphatase, alkaline phosphodiesterase and L-leucine-β-naphthylamidase, but the absence of intracellular enzymes.

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