The influence of some biological surfactants on the overturn time in goldfish

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Bile salts and phosphatides are the two major types of surface active compounds occurring naturally in the gastrointestinal tract. In the rat the absorption of some drugs is enhanced by the presence of bile salts (Kakemi, Sezaki & others, 1970; Kimura, Sezaki & Kakemi, 1972); similar results have been reported for the goldfish (Gibaldi & Nightingale, 1968; Nightingale, Wynn & Gibaldi, 1969; Marriott & Kellaway, 1976). Mayersohn, Feldman & Gibaldi (1969) demonstrated a 50–80% increase in riboflavin absorption in man when sodium deoxycholate was administered concomitantly.

In this work the action of sodium deoxycholate, sodium cholate and lysophosphatidylcholine on the gill membranes of the goldfish, *Carassius aurata*, is examined by observation of the overturn time. Fish used were all from the same batch and weighed 2–4 g. 100 ml of bathing solution (tris pH 7·0 buffer) was employed for each fish and none of the overturned fish were re-used. A minimum of 5 fish were used for each surfactant concentration examined.

Minimum effective concentrations were observed (Fig. 1) for both sodium deoxycholate (≃0·6 mM) and sodium cholate (≃5·8 mM); the greater toxicity of the dihydroxy compared with the trihydroxy salt is in agreement with the absorption promoting capacities (Marriott & Kellaway, 1976). The linear relation between T^{-1} and concentration when extrapolated to the abcissa yielded concentrations in agreement with the values for the critical micelle concentration (cmc) of 6 mM for sodium deoxycholate and 12 mM for sodium cholate (Norman, 1960). Minima occurred in the cmc region which suggests desorption of the bile salt from the membranes, possibly as a complex with a membrane component, which would subsequently be solubilized by the bile salt micelles. The effect below the cmc probably involves the incorporation of the bile salt molecules into the lipid bilayer, thus disrupting the natural molecular arrangement of the membrane to produce a structure across which drugs may be more rapidly transported (Marriott & Kellaway, 1976).

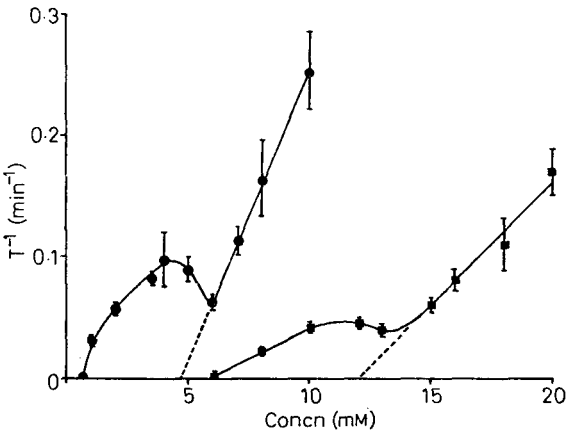

FIG. 1. The effect of bile salt concentration (mM) on the reciprocal overturn time, T^{-1} (min^{-1}) for sodium deoxycholate ● and sodium cholate ■.

The fish were susceptible to lower concentrations of bile salt than the fish from a different batch used earlier (Marriott & Kellaway, 1976). This interbatch variation has been observed previously (Nightingale & others, 1969).

The disruption of the membranes by lysophosphatidylcholine was dependent only on the concentration of the phosphatide present. Five concentrations were examined in the range 2–12 mM and the relationship between T^{-1} (min) and concentration (C) was $T^{-1} = 0{\cdot}0078\ C + 0{\cdot}00025$ ($r = 0{\cdot}996$). The cmc of lysophosphatidylcholine at 20° is reported to be $6{\cdot}1 \times 10^{-6}$ g cm^{-3} (Purdon, Tinker & Neumann, 1976) and $10{\cdot}1 \times 10^{-6}$ g cm^{-3} (Robinson & Saunders, 1958) both values being well below the concentration range examined in this study. All three surfactants examined, therefore, exhibit a linear response between T^{-1} and the post cmc concentration of the surfactant, although for lysophosphatidylcholine extrapolation of the data did not coincide with the literature cmc value. This is probably due to the low value of the cmc and experimental error.

The disaggregation of lipid-protein complexes requires substances such as bile salts and lysophatidylcholine which can compete for hydrophobic bonding sites on the protein molecules (Helenius & Simons, 1972; Makino, Reynolds & Tanford, 1973; Tanford, Nozaki & others, 1974). High concentrations of deoxycholate have been shown to remove lipids from proteins in the human red blood cell membrane (Philippot, 1971). Most extracted lipids are incorporated into mixed lipid-surfactant micelles. Hence bile salts in concentrations in excess of the cmc will extract and solubilize both protein and lipids from intact biological membranes. At concentrations below the cmc, the bile salt molecules are incorporated into membranes changing the permeability characteristics by a mechanism as yet unknown.

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Concentration of prolactin in serum of rats treated with baclofen

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Baclofen (Lioresal) has central depressant properties which are useful for the control of spasticity (Jones, Burke & others, 1970). The drug also appears to act like γ-hydroxybutyric acid and γ-butyrolactone to inhibit the firing of dopaminergic nerves in the nigrostriatal and mesolimbic neuronal systems, as evidenced by the ability of these drugs to block neuroleptic-induced increase of dopamine turnover (Fuxe, Hökfelt & others, 1975) and increase the concentration of dopamine in brain regions innervated by these neurons (Da Prada & Keller, 1976; Kelly & Moore, 1976; Gianutsos & Moore, 1977). To determine if baclofen has a similar action on dopamine neurons in the tuberoinfundibular system, the effects of this drug on serum prolactin concentrations was determined. Secretion of prolactin is tonically inhibited by tuberoinfundibular dopaminergic neurons so that drugs which interfere with the activity or actions of these neurons should cause the concentration of prolactin in the serum to increase (MacLeod, 1974).

Adult male Sprague-Dawley rats, 200–250 g, received injections of various doses of baclofen or saline vehicle intraperitoneally and were decapitated 1 h later. Blood collected from the trunk was centrifuged and serum stored at −20° until assayed for prolactin by the double antibody radioimmunoassay described by Niswender, Chen & others (1969). Values are expressed in terms of NIAMDD-rat prolactin-PR-1.

Baclofen at 5 and 10 mg kg^{-1} slightly reduced, but at 20 mg kg^{-1} markedly increased the serum concentration of prolactin. Since the animals are anaesthetized at the higher dose, and appear to have respiratory difficulties, the effect on prolactin may be unrelated to a specific action of the drug on tuberoinfundibular neurons, but rather to a non-specific stress-induced effect. Many types of stressful situations increase serum prolactin concentrations (Neill, 1974).

Neuroleptics increase serum concentrations of prolactin (Dickerman, Clark & others, 1972; Clemens, Smalstig & Sawyer, 1974), presumably due to their ability to block dopamine receptors. Fredericksen (1975) reported that baclofen is effective in treating some schizophrenics and may enhance the antipsychotic effects of neuroleptics, although others have been unable to confirm these effects (Simpson, Branchey & Shrivastava, 1976). Nevertheless, it was of interest to determine if baclofen enhanced the prolactin elevating action of neuroleptics. For this purpose, a dose of haloperidol which has been shown to be at the threshold for increasing serum prolactin concentrations (Mueller, Simpkins & others, 1976) was administered 2 h before the administration of a range of doses of baclofen having no influence on prolactin concentrations. It was postulated that the two drugs, acting together, would increase the serum concentration of prolactin, but as summarized in Table 1, this was not so. Haloperidol (0·03 mg kg^{-1}) pretreatment produced a slight increase in the circulating prolactin concentrations. In vehicle-pretreated rats 10 mg kg^{-1} or less of baclofen did not increase prolactin concentrations, confirming the results in Fig. 1. When increasing doses of baclofen were administered to rats pretreated with haloperidol the serum prolactin concentrations were not significantly different from values in animals pre-treated with the haloperidol vehicle.

Since non-anaesthetic doses of baclofen did not increase serum prolactin concentrations in the first experiment and since baclofen did not enhance the effects of haloperidol on prolactin concentrations in the second experiment, it would appear that, at least in non-anaesthetic doses, baclofen does not inhibit the firing of

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