J. Pharm. Pharmac., 1976, **28**, 620–624 *Received March* 1, 1976

A comparative study of the effect of bile salts on the absorption of quinalbarbitone sodium in goldfish

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The goldfish has been used as a model membrane to estimate the absorption of quinalbarbitone sodium by measurement of overturn time. The effect of sodium cholate, sodium deoxycholate, sodium chenodeoxycholate and sodium taurodeoxycholate on the quinalbarbitone-induced overturn time has been investigated and differences in capacity to promote absorption have been demonstrated. These differences could not be attributed to variations in solubility or partitioning of the drug within the membrane by the bile salts or to the relative hydrophobicity of each molecule. It is suggested that the bile salts must in someway affect the integrity of the goldfish membrane rendering it more permeable to quinalbarbitone sodium.

The bile salts or acids are one of the most important groups of surfactants which occur naturally in man. Although considerable progress has been made in the understanding of the luminal and cellular phases of fat absorption (Hofmann & Small, 1967), relatively little is known of the role played in the passive transport of drugs across lipid membranes. Since it is difficult to carry out such studies *in vivo* the goldfish has been widely used as a model system to investigate not only the effect of bile salts (Gibaldi & Nightingale, 1968; Nightingale, Wynn & Gibaldi, 1969) but also other surfactants (Levy, Miller & Reuning, 1966; Anello & Levy, 1969; Gouda, 1974; Florence & Gillan, 1975) and dimethylsulphoxide (Florence, 1975).

We now describe some results of experiments designed to compare the effect of four bile salts, present in man, on the absorption of quinalbarbitone sodium in goldfish. We have also examined some of the possible mechanisms of action of the bile salts.

MATERIALS AND METHODS

Goldfish, *Carassius aurata*, from a single batch, of 3–6 g, were used. The overturn time (T_0) was determined with individual fish immersed in 150 ml tris buffer (pH 7·0) at 18–20° and not less than five fish were used for each determination. The reciprocal of the overturn time was used as an index of drug absorption. Determinations with quinalbarbitone sodium (Eli Lilly & Co. Ltd., Basingstoke) were made at concentrations of up to 25 mg dl^{-1}. The effect of the bile salts sodium cholate (Sigma (London) Chemical Co. Ltd., Surrey) sodium deoxycholate (Koch Light Laboratories Ltd., Bucks), sodium chenodeoxycholate and taurodeoxycholate (Weddel Pharmaceuticals Ltd., London) at concentrations of up to 2 mM was determined on the absorption of the quinalbarbitone sodium (20 mg dl^{-1}).

The equilibrium solubility of quinalbarbitone sodium in varying concentrations of sodium cholate and deoxycholate was determined at 25° by weighing excess drug into 2 ml of tris buffer and allowing 48 h for equilibration to occur. The resultant suspensions were then centrifuged at 15 000 *g* for 30 min at 25° after which the clear supernatants were removed and diluted with equal parts of tris buffer and 0·5 M sodium hydroxide solution. This dilute solution was assayed for quinalbarbitone sodium at 255 nm.

The apparent partition coefficients were determined at 37° in the tris buffer–1-octanol system since 1-octanol has been suggested by Smith, Hansch & Amies (1975) as the most suitable lipophilic phase for such determinations. The quinalbarbitone sodium concentration was 4 mg dl^{-1} whilst the bile salt concentration varied from 0·1 to 1·0 g dl^{-1} in the aqueous phase, which consisted of tris buffer (pH 7·0) saturated with 1-octanol. The total volume of the aqueous phase was 10 ml and to this was added 10 ml of 1-octanol saturated with buffer. After shaking the solutions for 48 h the two phases were separated and the aqueous phase analysed for both bile salt and quinalbarbitone sodium. The bile salt was assayed by the addition of 0·1 ml of the aqueous solution to 10 ml of the Minibeck reagent (9 parts conc. sulphuric acid plus 1 part glacial acetic acid) and incubating at 40° for 90 min (Singer & Fitschen, 1961). The solution was then allowed

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to stand at room temperature for 30 min before the absorbance was determined at 390 nm.

The critical micelle concentration (cmc) of sodium deoxycholate was determined at 25° using a B642 Autobalance Universal Conductivity bridge in conjunction with a E7591/B dip cell (Wayne Kerr, New Malden, Surrey). Conductivities were determined for a range of bile salt concentrations in triple distilled water, tris buffer and tris buffer plus 20 mg % quinalbarbitone sodium. The cmc was taken as the break in the $\sqrt{M}$ *vs* molar conductivity plot.

The cmc was also determined by measurement of the surface tension of similar solutions to those above using a micro-force balance (C.I. Electronics, Salisbury, Wilts) in conjunction with 0·5 cm glass plate. The break in the concentration *vs* surface tension curve was again taken as the cmc.

Interfacial tension measurements with the four bile salts (0·5 mM in the aqueous phase) were made at the water–cyclohexane interface using the microforce balance in conjunction with a 1 cm glass plate. The water was triple distilled and the cyclohexane was spectroscopic grade (BDH Chemicals Ltd., Poole) and used as supplied.

RESULTS AND DISCUSSION

The relation between the reciprocal overturn time (T^{-1}) for the goldfish and the dose of quinalbarbitone sodium (5–25 mg dl^{-1}) added to the bathing solution was linear ($r = 0{\cdot}9997$) indicating obeyance of the Levy-Gucinski equation (Levy & Gucinski, 1964) although the threshold concentration reported by Nightingale & Gibaldi (1971) was not observed, presumably because the minimum concentration tested (5 mg dl^{-1}) was too high. The simple form of the equation is given by,

$$\frac{1}{T} = \frac{C_0 A D}{L} = \frac{K C_0}{L}$$

where C_0 = drug concentration outside the membrane, A = area of the membrane, D = diffusion coefficient, L = lethal dose of drug and K = the rate constant for absorption.

From the lethal dose for quinalbarbitone sodium given as 0·038 mg g^{-1} weight by Levy & others (1966), two values of K may be calculated. The first using the total drug concentration ($K_T = 9{\cdot}7 \times 10^{-6}$ 1 g^{-1} min^{-1}) and the second using only the concentration of unionized drug ($K_u = 11{\cdot}0 \times 10^{-6}$ 1 g^{-1} min^{-1}). The latter value compares favourably with that of $8{\cdot}19 \times 10^{-6}$ 1 g^{-1} min^{-1} reported by Levy & others (1966) particularly when it is considered that these workers used the actual death time of the goldfish rather than the overturn time and this would tend to increase the value of K_u.

Fig. 1 shows the effect of one of the bile salts, sodium deoxycholate, on the quinalbarbitone sodium-induced overturn time of the goldfish. A potentiation of effect between 0·04 and 0·7 mM bile salt concentration is observed. This is similar to the effect observed by Nightingale & others (1969) using sodium taurodeoxycholate and 4-aminoantipyrine

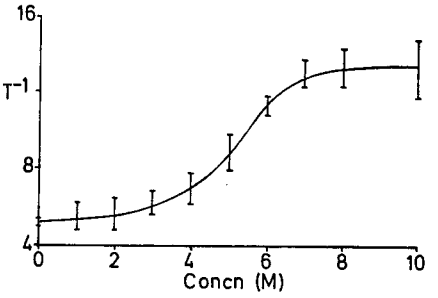

FIG. 1. The effect of sodium deoxycholate concentration ($\times 10^{-4}$ M) on the reciprocal overturn time (T^{-1}, $\times 10^{-2}$ min^{-1}) of goldfish.

although these workers found the effective range to be much narrower (approximately 0·8–1·0 mM). It has been suggested that the mechanism of action involves a rapid absorption of the bile salt on the external membranes of the fish with subsequent alteration of the membrane permeability (Gibaldi & Nightingale, 1968), but that a minimum surface concentration is required to elicit disruption of the membrane (Nightingale & others, 1969). The adsorption of bile salt molecules beyond this concentration would appear to have no effect although the maximum concentration used was only 1 mM, since concentrations of 3 mM bile salt have been shown to produce an obvious decrease in activity and occasional overturn (Nightingale & others, 1969).

The effect on overturn time of the four bile salts is shown in Fig. 2 where, for convenience, the bile salt concentration is represented logarithmically. The curves produced for two of the dihydroxy salts—deoxycholate and chenodeoxycholate—are similar, particularly in the case of the concentration which produced the maximum effect. The taurodeoxycholate, which differs only in that it is a conjugated salt, produced a similar maximum effect although it appears to be more potent at lower concentrations. In contrast, the trihydroxy salt, sodium cholate, produces a much lower maximum effect. The order of potency would therefore appear to be sodium

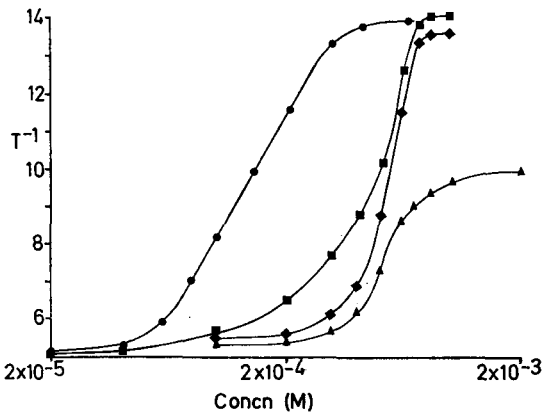

FIG. 2. The effect of bile salt concentration on the reciprocal overturn time (T^{-1}, $\times 10^{-2}$ min^{-1}) for goldfish. ▲ Sodium cholate, ◆ Sodium deoxycholate, ■ Sodium chenodeoxycholate, ● Sodium taurodeoxycholate.

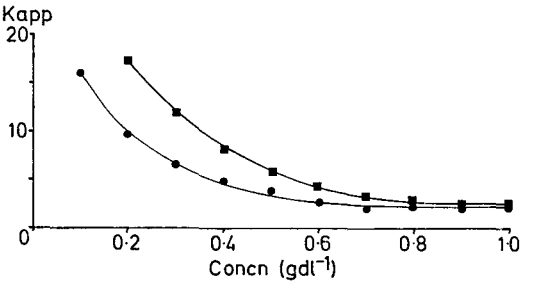

FIG. 3. The variation of the apparent partition coefficient (K_{app}) with bile salt concentration. ■ Sodium deoxycholate, ● Sodium cholate.

taurodeoxycholate > sodium chenodeoxycholate ⩾ sodium deoxycholate > sodium cholate.

A number of suggestions may be made to explain the difference in the activity of the various bile salts. It is possible that the action of the quinalbarbitone is potentiated by the depressant effect of the bile salts alone. Although this proposal may not be completely eliminated it is unlikely to be solely responsible for the observed results since the bile salt concentrations used in this work were shown to be ineffective by themselves. Also, Gibaldi & Nightingale (1968) observed that pretreatment of the goldfish with sodium taurodeoxycholate produced a similar overturn time on subsequent treatment with pentobarbitone to that obtained when the drug and bile salt were added simultaneously.

Another possible mode of action would be the induction of an alteration in the solubility or partitioning of the quinalbarbitone within the membrane by the bile salt. The equilibrium solubility determinations indicated that with sodium deoxycholate and cholate no changes in solubility were observed with bile salt concentrations of up to 0·5 g dl^{-1}. This would be predicted for a drug which is as highly water soluble as sodium quinalbarbitone since any changes induced by the bile salts are likely to be insignificant.

The change in apparent partition coefficient with concentration for sodium cholate and sodium deoxycholate are shown in Fig. 3 and values for the four bile salts at a concentration of 0·25 g dl^{-1} in tris buffer-1-octanol are sodium taurodeoxycholate 1·83, sodium cholate 7·52, sodium deoxycholate 14·74 and sodium chenodeoxycholate 23·46. With the exception of the conjugated bile salt, pH 7·0 is close to the pKa for the molecule (Ekwall, Rosendahl & Lofman, 1957), and therefore the values reflect an equilibrium between the ionized and unionized species over a period of time. The apparent partition coefficient decreases over the concentration range investigated and this indicates a reduced tendency to concentrate in the lipid phase at higher concentrations. This effect is most probably due to the change in pH (e.g. 1·0 g dl^{-1} sodium deoxycholate changes the pH from 7·00 to 8·01) which results from the addition of the bile salt. The observed behaviour would not explain the potentiation of the quinalbarbitone effect since the increase in pH would result in a decrease in the amount of unionized drug and this would not favour absorption. Also, there is no correlation between the order of effectiveness of the bile salts and the apparent partition coefficients. Further evidence is provided by the apparent partition coefficients of the quinalbarbitone sodium in the presence of up to 0·5 g dl^{-1} of sodium cholate and deoxycholate. Again the partition coefficient decreases from around 3·5 at 0·1 g dl^{-1} with increasing bilt salt concentration to a K_{app} of 2·0–2·3 at 0·5 g dl^{-1} which would be expected to produce less favourable conditions for absorption. It is of particular importance to note that the maximum concentration of bile salt used for the goldfish experiments would not be high enough to induce any changes in partitioning of the drug. Also, the maximum pH change produced by the addition of the bile salt was only 0·045 pH units.

Since non-ionic surfactants have been shown to increase drug absorption in the goldfish when the cmc is approached (Levy & others, 1966), it is feasible that, although the bile salt concentration used was well below the cmc, the commencement of

the upper plateau region (maximum 1/T) may arise from the lowering of the cmc due to the presence of buffer and drug. Table 1 shows the cmc for sodium deoxycholate in water, tris buffer and in the presence of drug. Although the cmc has been lowered, the concentration is still four times higher than that used in the goldfish studies. Hence, the observed effect cannot be attributed to the decrease in thermodynamic activity of the bile salts at the cmc.

Table 1. *Critical micelle concentration (cmc) of sodium deoxycholate in different solvent systems determined by conductivity and surface tension measurements.*

Solvent system used to dissolve sodium deoxycholate	cmc (mM) Conductivity	cmc (mM) Surface tension
Triple distilled water	5·04	4·73
Tris buffer (pH 7)	4·76	4·15
Tris buffer (pH 7) + 0·02 g dl^{-1} quinalbarbitone sodium	4·49	4·05

The value of the cmc in water is in agreement with the value of 4–6 mM quoted by Hofman & Small (1967). The higher values obtained by conductivity measurements have been observed by other workers (Ekwall, 1954; Ekwall, Fontell & Sten, 1957; Norman, 1960). The latter worker reported a value of 5 mM for sodium deoxycholate.

The decrease in interfacial tension π (mN m^{-1}) at the water–cyclohexane interface produced by 0·5 mM bile salt in the aqueous phase were: sodium chenodeoxycholate 36·4, sodium deoxycholate 33·9, sodium taurodeoxycholate 32·2 and sodium cholate 31·2, indicating that a decrease in interfacial tension is produced by all four bile salts. The concentration of 0·5 mM was selected since at this concentration a graded response for the bile salts had been obtained in the goldfish studies. No direct correlation was obtained between the rank order for decrease in interfacial tension and effectiveness as absorption promoters. Neither does this latter property appear to be a function of the relative hydrophobicity of the bile salts as there is also a lack of correlation between the rank order for the partition coefficients and the absorption data.

A number of workers have demonstrated an increased absorption in the presence of bile salts and other surfactants (Gibaldi & Nightingale, 1968; Anello & Levy, 1969; Nightingale & others, 1969; Florence & Gillan, 1975) and it is suggested that when an increase in permeability occurs then a specific effect on the lipid membrane is involved (Cuthbert, 1967). This action of bile salts would have been predicted since it has been reported that relatively small concentrations were capable of releasing lipid from erythrocyte ghosts (Kamat & Chapman, 1968) and Small, Penkett & Chapman (1969) found that bile salts converted the broad spectrum of lecithin in D_2O to a high resolution nmr spectrum. This indicates the ability of bile salts to break intermolecular hydrogen bonds as well as to disrupt the tight packing of hydrophobic regions (Hofmann & Small, 1967). It does seem feasible, therefore that the bile salts used in this work do affect the integrity of the goldfish membrane, rendering it more permeable to quinalbarbitone sodium. However, this effect also appears to be reversible since the fish displayed a similar overturn time if left for 48 h between experiments. Although it is probably unreasonable to extrapolate the results of this work to the *in vivo* situation it does suggest that the membranes of the gastrointestinal tract would be rendered more permeable to drug molecules by the action of the bile salts. This suggestion has been substantiated by the work of Kakemi, Sezaki & others (1970) and Kimura, Sezaki & Kakemi (1972) on the effect of bile salts on drug absorption from the rat gastrointestinal tract.

However, whatever their mode of action, the different bile salt molecules have been shown in this work to vary in their capacity to assist the passage of drugs across membranes.

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