Biochimica et Biophysica Acta, 640 (1981) 55—65

BBA 79040

MEMBRANE FLUIDITY AND BILE SALT DAMAGE

PHILIP J. LOWE and ROGER COLEMAN *

Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT (U.K.)

(Received June 27th, 1980)

Key words: Bile salt; Membrane damage; Lysis; Fluidity; Choline-containing phospholipid; (Erythrocyte)

Summary

The lysis, by bile salts, of membranes of different fluidities was studied; it was shown that membranes of low fluidity were less readily lysed than membranes of higher fluidity.

Membrane fluidity levels were controlled (i) by the use of erythrocytes, from different species, systematically differing in their lipid composition; (ii) by using each membrane at a range of temperatures; and (iii) by incorporating into the membranes the fluidizing agent, benzyl alcohol, at a range of concentrations. Membrane fluidity (and order) in each case was monitored by measuring the degree of polarization of fluorescence from the hydrophobic probe molecule, 1,6-diphenyl-1,3,5-hexatriene.

The response of lytic behaviour to modulations of membrane fluidity also indicated a difference between the bile salts, glycodeoxycholate and glycocholate; the former initiates lysis close to (at or below) its critical micellar concentrations whereas the latter only causes lysis above, and often substantially above, its critical micellar concentration. In their respective ranges of lytic concentrations, both bile salts are far less effective with membranes of low fluidity.

The results are discussed with regard to the features of a membrane which would be expected to be resistant to high concentrations of bile salts in vivo, i.e., the plasma membranes of the bile canaliculus and lumenal surface of biliary tract cells.

* To whom correspondence should be addressed.
Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

Introduction

Previous studies have shown that erythrocyte membranes differ in their susceptibility to damage by the same bile salt [1,2]; thus, erythrocytes with a high mole fraction of sphingomyelin (of the total choline phospholipids) were much less susceptible to lysis by sodium glycocholate than erythrocytes with a high mole fraction of phosphatidylcholine. Since sphingomyelin is generally less fluid than phosphatidylcholine [3,4], it is possible that the difference in susceptibility to bile salt attack may relate to differences in membrane fluidity, rather than to the mole fractions of the choline-containing phospholipids per se.

Borochov and co-workers [5] have shown that decreasing the sphingomyelin mole fraction in sheep erythrocyte membranes increases the fluidity of the membrane; these erythrocytes are then more susceptible to lysis by glycocholate when compared to appropriate controls [2].

In the present experiments, a series of erythrocytes was selected to represent significant variations in the sphingomyelin mole fraction (Table I). The susceptibility of these erythrocytes to lysis by bile salts was investigated (i) at decreasing temperatures, to increase the rigidity of the membrane [4], and (ii) in the presence of benzyl alcohol, to fluidize the membrane [7,8].

The fluidity of the membranes was estimated by measuring the degree of polarisation of the fluorescence from the probe molecule, 1,6-diphenyl-1,3,5-hexatriene; this contains information on the relaxation time (freedom) of the probe as it moves within a 'cone' and also the static orientational constraint of the probe. Thus, the 'fluidity' measurement by fluorescence polarisation is a reflection of both the dynamic state and the order of the membrane components [9—11].

Two bile salts were used in the study — sodium glycocholate, a trihydroxy bile salt, and sodium glycodeoxycholate, a dihydroxy bile salt — and their effects compared.

A preliminary account of some of this work has appeared [12].

TABLE I

CHOLINE-CONTAINING PHOSPHOLIPIDS OF ERYTHROCYTE MEMBRANES FROM VARIOUS MAMMALIAN SPECIES

The relative amounts of sphingomyelin and phosphatidylcholine are expressed as a percentage of the total membrane phospholipids. (From White [6].)

Species	% of total phospholipids		Sphingomyelin mole fraction of total choline phospholipids
	Sphingomyelin	Phosphatidylcholine	
Guinea-pig	11	41	0.21
Rat	13	48	0.21
Human	20	35	0.37
Pig	27	23	0.53
Ox	46	4	0.92
Sheep	51	1	0.98

Materials and Methods

Materials

Ox, sheep and pig blood from local slaughterhouses, and blood from guinea-pigs (female, approx. 450 g) and rats (male, approx. 250 g) were taken into 0.33 vol. of acid citrate/dextrose solution. Human blood in acid citrate/dextrose solution was obtained by courtesy of a local transfusion service and was used within 7 days of donation. Erythrocytes were washed (three times, 5 vol.) and finally resuspended in 0.154 M NaCl, 1.5 mM Hepes (adjusted to pH 7.4), to a final concentration of approx. 2 μmol phospholipid phosphorus per ml (assayed according to the method of Bartlett [13]).

Erythrocyte ghosts were prepared by haemolysis and subsequent washing with a 40 imosM solution containing 18 mM NaCl, 1.5 mM Hepes, 1 mM EDTA (adjusted to pH 7.4) [14]. The packed ghosts were assayed for phospholipid and diluted with appropriate volumes of 18 mM NaCl, 1.5 mM Hepes, 1 mM EDTA (pH 7.4). Glycocholate and glycodeoxycholate (sodium salts, A grade, more than 98% pure) were obtained from Calbiochem Ltd., Bishops Stortford, Herts, U.K. 1,6-Diphenyl-1,3,5-hexatriene was obtained from Aldrich Chemical Co. Ltd., Gillingham, Dorset, and benzyl alcohol from Fisons, Loughborough, Leics. All other fine chemicals were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K.

Methods

Haemolysis of erythrocytes by bile salts. 1 vol. of the washed erythrocyte suspension was incubated for 10 min with 3 vol. of 0.14 M NaCl, 15 mM Hepes (pH 7.4) containing appropriate concentrations of bile salts. Incubations were terminated by centrifugation at 14 000 × *g* for 1 min (Jobling 320 microcentrifuge). The percentage haemolysis was determined by comparing the absorbance at 525 nm of appropriate dilutions of the supernatant with that of an uncentrifuged control totally haemolysed by dilution to 20 vol. with water.

Membrane fluidity measurements. The fluidity of the membrane lipid core was measured by a fluorescence polarisation technique as outlined by Shinitzky and coworkers [4,15]. A suspension of diphenylhexatriene (2 μM) was prepared freshly by adding a known volume of 1 mM diphenylhexatriene (in tetrahydrofuran) to an appropriate volume of 18 mM NaCl, 1.5 mM Hepes, 1 mM EDTA (pH 7.4) whilst vortex mixing. To 1 vol. of the 2 μM diphenylhexatriene suspension was added 1 vol. of ghosts (0.2 μmol/ml phospholipid phosphorus); the mixture was incubated at 37°C for 30 min. The degree of polarisation of fluorescence from the probe was then measured in an apparatus based on the design of Weber and Bablouzian [16], modified according to Teale [17]. The excitation wavelength was 360 nm; fluorescence was detected at 430 nm with a filter to cut off wavelengths below 405 nm. The fluorescence polarisation was measured between 37 and 5°C and then between 5 and 37°C, in steps of 5°C (values at each temperature were averaged), using a jacketed cell and pumping water bath. The sample was left at each temperature until the reading of fluorescence polarisation had stabilized.

Benzyl alcohol experiments. Haemolysis experiments were carried out by

adding 1 vol. of washed erythrocyte suspension to 3 vol. of 0.14 M NaCl, 15 mM Hepes (pH 7.4) containing appropriate concentrations of bile salts and benzyl alcohol. The mixture was incubated at 37°C (sheep erythrocytes), or at 25°C (human erythrocytes), for 10 min and then centrifuged; the degree of haemolysis was estimated as above.

Fluorescence polarization experiments were carried out following preincubation to allow the probe to equilibrate into the membrane; the membrane was then exposed to benzyl alcohol and the fluorescence polarization measured.

A suspension of diphenylhexatriene (20 μM in 18 mM NaCl, 1.5 mM Hepes, 1 mM EDTA, pH 7.4) was freshly prepared as above. To 1 vol. of this was added 1 vol. of ghosts (2 μmol/ml phospholipid phosphorus); the mixture was incubated at 37°C for 30 min. 0.2 ml of this was added to 1.8 ml of an appropriate concentration of benzyl alcohol in 18 mM NaCl, 1.5 mM Hepes, 1 mM EDTA (pH 7.4), which had been pre-warmed in the fluorescence cuvette; the fluorescence polarisation was read after 1 min. It was noted that the fluorescence polarisation in the presence of benzyl alcohol was established within 30 s, and then remained constant for longer than 30 min. Diphenylhexatriene does not fluorescence significantly in aqueous benzyl alcohol.

Preparation and use of liposomes. Lipid extracts of human or sheep ghosts were prepared according to the method of Bligh and Dyer [18]. The extracts were dried down in vacuo and the lipids were then dissolved in chloroform/ether (1 : 2, v/v) to give a solution of approx. 2 μmol/ml. This solution was then aspirated through a fine-drawn capillary (bore 50–100 μm) into 18 mM NaCl, 1.5 mM Hepes, 1 mM EDTA (pH 7.4) which was maintained at 50°C. The liposome suspension produced was then passed through a 0.8 μm Millipore filter, assayed for phospholipid phosphorus and diluted to 0.2 μmol/ml phospholipid phosphorus. The method used for the preparation was essentially that of Deamer and Bangham [19], modified to counteract the relative insolubility of sphingomyelin in ether by the use of chloroform/ether; this then necessitated the higher temperature during aspiration.

Fluorescence polarisation experiments were carried out as above, substituting the liposome preparation for the erythrocyte ghosts suspension.

Critical micellar concentration. This was determined for glycocholate and for glycodeoxycholate, separately, in 0.14 M NaCl, 15 mM Hepes (pH 7.4), by using the method of Benzonana [20].

Results

Effect of temperature on bile salt-induced lysis

The influence of membrane lipid composition upon erythrocyte lysis is shown in Fig. 1 (for glycocholate lysis) and in Fig. 2 (for glycodeoxycholate lysis). In both cases, the increasing proportion of sphingomyelin was associated with a flattening of the lysis curve (e.g., at 37°C). In the case of glycocholate, there was also a substantial shift in the inflection point of the lysis curve (lysis point) towards higher concentrations with membranes of higher sphingomyelin mole fraction; thus, 10% lysis of rat and guinea-pig erythrocytes (sphingomyelin mole fraction 0.21) required 10 mM glycocholate whereas 10% lysis of

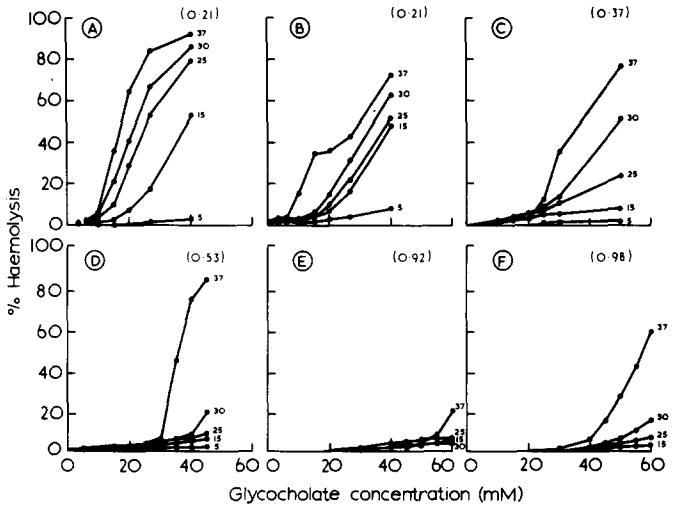

Fig. 1. Haemolysis of erythrocytes by glycocholate at different temperatures. For experimental details see Materials and Methods. Figures on individual curves refer to temperature of incubation in °C. Numbers in parentheses refer to mole fraction of sphingomyelin of total choline-containing phospholipids. (A) Guinea-pig, (B) rat, (C) human, (D) Pig, (E) ox, (F) sheep. Values represent means of two experiments at each temperature.

ox and sheep erythrocytes (sphingomyelin mole fraction 0.92—0.98) required 35—50 mM glycocholate.

With glycocholate, lowering the temperature of incubation in all cases reduced the extent of haemolysis (Fig. 1). The effect of decreasing temperature

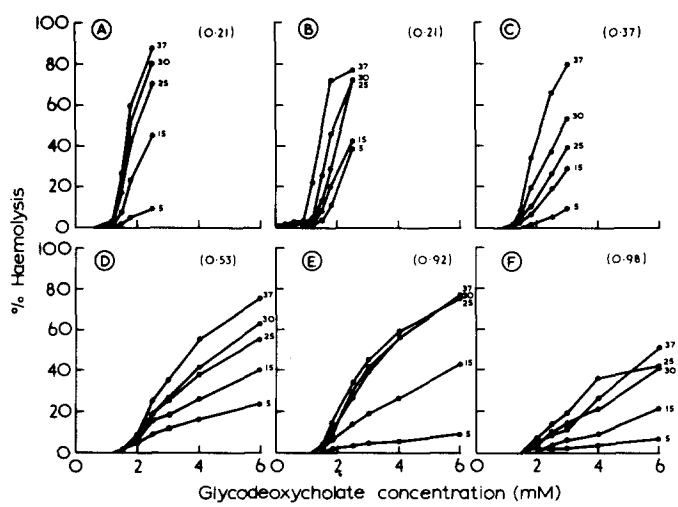

Fig. 2. Haemolysis of erythrocytes by glycodeoxycholate at different temperatures. For experimental details see Materials and Methods. Figures on individual curves refer to temperature of incubation in °C. Numbers in parentheses refer to mole fraction of sphingomyelin of total choline-containing phospholipids. (A) Guinea-pig, (B) rat, (C) human, (D) pig, (E) ox, (F) sheep. Values represent means of two experiments at each temperature.

was both to flatten the lysis curve and to shift the lysis point towards higher bile salt concentrations; e.g., for rat erythrocytes (sphingomyelin mole fraction 0.21), 10% lysis at 37°C required 10 mM glycocholate whereas at 5°C it required approx. 35 mM. With glycodeoxycholate, the effect of decreasing temperature was to flatten the lysis curve; there was little or no effect upon the position of the lysis point. The patterns of the lysis response to temperature were reminiscent, therefore, of the patterns of the lysis response to lipid composition.

The critical micellar concentrations for the bile salts, in the solutions used, were 6.1 mM for glycocholate and 1.7 mM for glycodeoxycholate; the critical micellar concentration did not vary significantly with temperature in the range 5—37°C. This shows that the effect of temperature on lysis was likely to be upon the membrane, rather than on the physical nature of the bile salts.

Estimation of membrane fluidity

At a given temperature, ghosts with a high mole fraction of sphingomyelin showed an increased degree of fluorescence polarization, i.e., were less fluid, compared to those with lower relative concentrations of sphingomyelin (Fig. 3A). When the temperature of any one species of erythrocyte ghosts containing diphenylhexatriene was decreased, the degree of polarization of the fluorescence increased, indicating a reduction in the fluidity of the membrane (Fig. 3A).

Haemolysis and fluidity of membranes in the presence of benzyl alcohol

The presence of benzyl alcohol induced an increase in susceptibility to lysis by both glycocholate and glycodeoxycholate (Fig. 4); the slope of the haemolysis curve was increased and, for glycocholate lysis, the inflection point was moved substantially towards lower bile salt concentrations. Benzyl alcohol alone did not lyse the erythrocytes in the concentration range used.

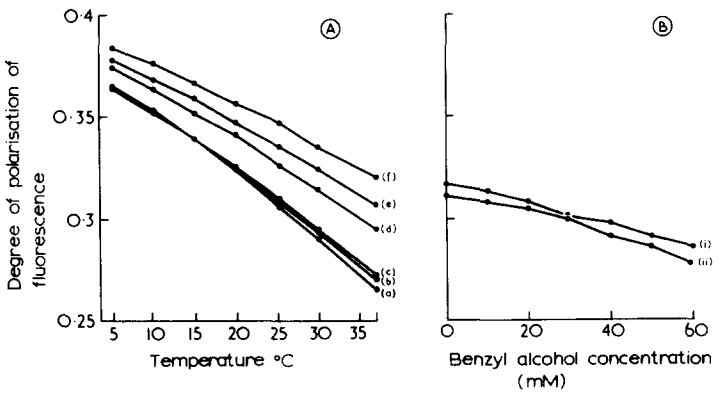

Fig. 3. Degree of polarization of fluorescence of diphenylhexatriene in erythrocyte membranes. (A) Variation with temperature. For experimental details see Materials and Methods. a, guinea-pig (sphingomyelin mole ratio 0.21); b, rat (0.21); c, human (0.37); d, pig (0.53); e, ox (0.92); f, sheep (0.98). Values represent means of two experiments. (B) Variation with benzyl alcohol concentration. For experimental details see Materials and Methods. (i) Human erythrocyte ghosts, temperature 25°C; (ii) sheep erythrocyte ghosts, temperature 37°C. Values represent means of two experiments.

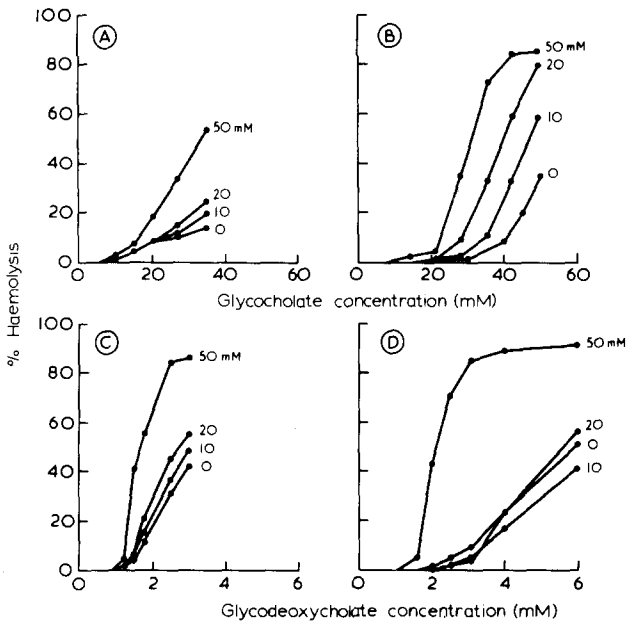

Fig. 4. Haemolysis of erythrocytes by bile salts in the presence of benzyl alcohol. For experimental details see Materials and Methods. Figures on individual curves represent concentration of benzyl alcohol present. A and C, human erythrocytes, temperature 25°. B and D, sheep erythrocytes, 37°C.

These experiments and those given in Fig. 3B were conducted with membranes which were initially both of low susceptibility to lysis and of low fluidity; in this way any increase could readily be seen. The membranes used were therefore those of sheep erythrocytes (sphingomyelin mole fraction 0.98) at 37°C and human erythrocytes (sphingomyelin mole fraction 0.37) at 25°C.

Measurements of the degree of fluorescence polarization (Fig. 3B) showed that when benzyl alcohol was added to the ghost preparations the degree of polarization fell, suggesting that the presence of benzyl alcohol had produced a more fluid membrane. As with the increase in susceptibility to haemolysis (Fig. 4) the effects were dose dependent.

TABLE II

POLARISATION OF FLUORESCENCE FROM DIPHENYLHEXATRIENE IN ERYTHROCYTE GHOSTS AND IN LIPOSOMES

For experimental details see Materials and Methods. The polarisation of fluorescence was measured at 37°C. Values are means ± S.E.; the numbers of experiments are in parentheses.

Species	Sphingomyelin mole fraction	Polarisation of fluorescence	
		Ghosts	Liposomes
Human	0.37	0.271 ± 0.001 (5)	0.280 ± 0.004 (3)
Sheep	0.98	0.318 ± 0.002 (7)	0.322 ± 0.001 (3)

Fluidity of liposomes made from lipids of different sphingomyelin mole ratios

Liposomes were made from total lipid extracts of both human and sheep erythrocyte membranes. The lipid extracts differed slightly in polarization from the original membranes (Table II), but it is clear that the lipids of sheep erythrocyte membranes (sphingomyelin mole fraction 0.98) were considerably less fluid than those of human erythrocyte membranes (sphingomyelin mole fraction 0.37).

Discussion

Bile salt lysis and membrane fluidity

Other studies have shown that the relative proportions of sphingomyelin and phosphatidylcholine in membranes are a determinant of membrane fluidity [3—5]. In phospholipid bilayers, sphingomyelin is less fluid than phosphatidylcholine for several reasons [3]: (i) sphingomyelin has a free hydroxyl group which can hydrogen bond to the amide group on neighbouring sphingomyelin molecules and can also make the molecule rigid internally by hydrogen bonding to the phosphate moiety; (ii) natural sphingomyelins generally have longer and more saturated fatty acids than natural phosphatidylcholines, thus their fatty acids can both project further into the opposite leaflet and pack closer together; and (iii) the presence of a *trans* double bond in the sphingosine chain also increases the rigidity of the molecule.

Lowering the temperature reduces membrane fluidity; diphenylhexatriene can detect the decrease in fluidity in the membrane core as its motion is more restricted [4]. At a given temperature, the partitioning of benzyl alcohol into the membrane increases membrane fluidity due to a general increase in the freedom of lipid movement; this has been detected by fatty acid spin labels [21] and by diphenylhexatriene (present experiments) reporting on the membrane core, by a change in the ^{1}H-NMR signal of the choline *N*-methyl groups reporting on head group movement [7] and by an increased translational movement of proteins embedded in lipid in the core [8].

The experiments described above (Results, Figs. 1—4) establish, for a given bile salt at a given concentration, that the extent of membrane lysis correlates, in general, with the degree of membrane fluidity. Essentially parallel effects on the extent of lysis are seen with the alterations in membrane fluidity resulting from changes in membrane lipid composition, from the effects of temperature and from the presence of benzyl alcohol, i.e., the more fluid the membrane the more susceptible it is to lysis. This conclusion is based upon the use of changes in the value of fluorescence polarisation as a comparative or correlative technique. Similar values of fluorescence polarisation, however, do not necessarily equate with an identical level of haemolysis in different membranes at a given bile salt concentration. The reasons for such anomalies are not known, but whilst it is justifiable to study changes in fluorescence polarisation as an indication of relative fluidity, the use of fluorescence polarization as an absolute technique to obtain individual values is less justifiable; individual value may have been influenced by such factors as local probe concentrations and local alterations in membrane composition.

The studies with liposomes derived from the lipids of different erythrocyte

membranes (Fig. 4) indicate that the membrane lipids substantially determine the fluidity characteristics of the membranes and may therefore influence the response of the membrane to the lytic action of bile salts. Direct studies of the lytic behaviour of glycocholate and taurocholate towards liposomes of different fluidities support this [22].

Differences in lytic behaviour between glycodeoxycholate and glycocholate

Glycodeoxycholate and glycocholate lysis of erythrocytes differ; glycodeoxycholate lysis occurs at a much lower bile salt concentration than glycocholate lysis and, for membranes of different fluidities, over a much narrower concentration range.

These differences cannot be explained only by the differences in critical micellar concentration between the two bile salts since (i) glycodeoxycholate lyses erythrocytes at or below its critical micellar concentration, whereas glycocholate lyses erythrocytes substantially above its critical micellar concentration; (ii) the point of lysis of erythrocytes by glycodeoxycholate is scarcely affected by the fluidity of the membrane, compared to lysis by glycocholate, where there is a marked effect.

Both bile salts can release components from membranes at concentrations below the point of lysis [2,23]; the release of components has been most extensively studied with glycocholate. It has been shown that two phenomena are possible: (i) the release of small vesicles containing most of the phospholipid types in the membrane and representing lipids from both leaflets of the membrane, together with a selected complement of membrane proteins [23]; (ii) the release of mixed micelles of the bile salt with membrane phospholipid, the composition of these is specialized and represents a disproportional contribution of lipids from the outer leaflet of the membrane [23,24].

With membranes of a low sphingomyelin mole fraction, i.e., of high fluidity, the release of microvesicles predominates and the contribution of mixed micelles is seen only at higher bile salt concentrations; with membranes of high sphingomyelin mole fraction, i.e., of low fluidity, the ability to release microvesicles is reduced and the lipid released is predominantly in mixed micelles [2].

Membrane lysis probably involves the penetration of the bile salt into the membrane and a subsequent modification of membrane structure in both leaflets of the bilayer. For lysis to occur prior to the critical micellar concentration of the bile salt, it is clear that the affinity of the bile salt for the membrane must be high compared to the affinity of the bile salt for itself; this is clearly the case for glycodeoxycholate. For glycocholate, a bile salt with less lipophilic character, it is clear that very high concentrations, often several times higher than the critical micellar concentration, can coexist with the membrane. This is particularly so with membranes of low fluidity. These observations suggest that the uptake of glycocholate is probably low, is probably regulated by the fluidity of the membrane, and may be largely restricted to the outer leaflet of the membrane. Beyond the lysis point, both leaflets of the membrane are probably perturbed and for both bile salts this perturbation is clearly regulated by the fluidity of the membrane.

Susceptibility of membranes to bile salt damage in vivo

Rat bile, collected by cannulating the common bile duct, contains, initially, approx. 30 mM bile salts. The major bile salts are cholate conjugates (approx. 12 mM) but deoxycholate conjugates are present at a concentration of about 5 mM [25]. Gall bladder bile from various animals is more concentrated; the principal bile salt of rabbit bile is glycodeoxycholate and this may rise to a concentration of approx. 300 mM [26]. How then do the cells of the liver and biliary tract survive exposure to such high concentrations of bile salts?

Bile is a multicomponent solution of bile salts, phospholipids, cholesterol and proteins. Phospholipid and cholesterol occur in mixed micelles with the bile salts [27] and might therefore attenuate the action of the bile salts on plasma membranes in the bile canaliculus and biliary tract [26]. Such attenuation by phospholipids and cholesterol (and possibly protein) may not be the only mechanism of preventing membrane damage within the biliary tract, since it has been shown that bile from various animals can be lytic to erythrocyte membranes at concentrations below which its bile salts occur in the biliary tract in vivo [26]. One further way for biological protection to work, therefore, is in the molecular design of the bile canalicular membrane, and of other plasma membranes in the biliary tract exposed to high concentrations of bile salts.

The model experiments presented in this paper indicate that membranes of low fluidity are less susceptible to lytic damage than membranes of higher fluidity. Thus, if the bile canaliculus and biliary tract lining membranes were of low fluidity they would be better able to resist the cytolytic action of bile salts. Preparations of liver plasma membrane containing bile canaliculi have relatively low fluidity values measured by fluorescence polarization using diphenylhexatriene [28] and by ESR using a nitroxide spin label in the hydrophobic region [29]. Unfortunately, in both of these studies, values of other liver membranes were not presented for comparison, nor was the plasma membrane preparation specifically purified to contain only canaliculus membranes. Plasma membrane preparations specifically purified or subfractionated to be enriched in canalicular membranes have been shown to contain a higher sphingomyelin mole fraction than other plasma membranes or cytoplasmic membranes [30,31]. Whether the high sphingomyelin content is the only mechanism for assuring a low fluidity and therefore, potential resistance to bile salts, remains to be determined.

Acknowledgements

We thank Dr. F.W.J. Teale for help and advice with fluorescence polarization, and Drs. M. Houslay and A. Watts for helpful discussion.

References

1 Coleman, R. and Billington, D. (1979) Biochem. Soc. Trans. 7, 948
2 Coleman, R., Lowe, P.J. and Billington, D. (1980) Biochim. Biophys. Acta 599, 294—300
3 Schmidt, C.F., Barenholz, Y. and Thompson, T.E. (1977) Biochemistry 16, 2649—2656
4 Shinitzky, M. and Barenholz, Y. (1974) J. Biol. Chem. 249, 2652—2658
5 Borochov, H., Zahler, P., Wilbrandt, W. and Shinitzky, M. (1977) Biochim. Biophys. Acta 470, 382—388

6 White, D.A. (1973) in Form and Function of Phospholipids (Ansell, B., Dawson, R.M.C. and Hawthorne, J.N., eds.), p. 454, Elsevier, Amsterdam
7 Colley, C.M. and Metcalfe, J.C. (1972) FEBS Lett. 24, 241—246
8 Dipple, I. and Houslay, M.D. (1978) Biochem. J. 174, 179—190
9 Hildenbrand, K. and Nicolau, C. (1979) Biochim. Biophys. Acta 553, 365—377
10 Heyn, M.P. (1979) FEBS Lett. 108, 359
11 Jähnig, F. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 6361—6365
12 Coleman, R. and Lowe, P.J. (1980) Biochem. Soc. Trans. 8, 126—127
13 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466—468
14 Bramley, T.A. and Coleman, R. (1972) Biochim. Biophys. Acta 290, 219—228
15 Shinitzky, M., Dianoux, A.C., Gitler, C. and Weber, G. (1971) Biochemistry 10, 2106—2113
16 Weber, G. and Bablouzian, B. (1966) J. Biol. Chem. 241, 2558—2561
17 Teale, F.W.J. (1970) J. Phys. E: 3, 555—557
18 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911—919
19 Deamer, D. and Bangham, A.D. (1976) Biochim. Biophys. Acta 443, 629—634
20 Benzonana, G. (1969) Biochim. Biophys. Acta 176, 836—848
21 Boggs, J.M., Yoong, T. and Hsia, J.C. (1976) Mol. Pharmacol. 12, 127—137
22 Richards, M.H. and Gardner, C.R. (1978) Biochim. Biophys. Acta 543, 508—522
23 Billington, D. and Coleman, R. (1978) Biochim. Biophys. Acta 509, 33—47
24 Billington, D., Coleman, R. and Lusak, Y.A. (1977) Biochim. Biophys. Acta 466, 526—530
25 Kakis, G. and Yousef, I.M. (1978) Can. J. Biochem. 56, 287—290
26 Coleman, R., Iqbal, S., Godfrey, P.P. and Billington, D. (1979) Biochem. J. 178, 201—208
27 Carey, M.C. and Small, D.M. (1970) Am. J. Med. 49, 590—608
28 Keefe, E.B., Scharschmidt, B.F., Blankenship, N.M. and Ockner, R.K. (1979) J. Clin. Invest. 64, 1590—1598
29 Slater, T.F., Ingall, A., Le Page, R.N. and Lott, K.A.K. (1979) in Biological Effects of Bile Acids (Paumgartner, G., Stiehl, A. and Gerok, W., eds.), pp. 19—24, MTP Press, Lancaster, U.K.
30 Kremmer, T., Wisher, M.H. and Evans, W.H. (1976) Biochim. Biophys. Acta 455, 655—664
31 Yousef, I.M., Bloxam, D.L., Phillips, M.J. and Fisher, M.M. (1975) Can. J. Biochem. 53, 989—997