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Cell Type–Dependent Effect of Phospholipid and Cholesterol on Bile Salt Cytotoxicity

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The effect of phosphatidylcholine and cholesterol on bile salt–induced cytotoxicity was investigated. Experiments were performed in both human erythrocytes and cultured CaCo-2 cells, a model system for gastrointestinal epithelium. Hemolysis induced by 50 mmol/L sodium-taurocholate was reduced by both lecithin and cholesterol in a concentration-dependent manner. Cholesterol was 10 times more effective than phosphatidylcholine. Addition of only small amounts of the sterol to phosphatidylcholine/taurocholate solutions eliminated all cytotoxicity. The protective influence of cholesterol is probably mediated through a direct effect on the membrane. Incubation of erythrocytes with a cholesterol/taurocholate mixture greatly increased the cholesterol content of the membrane. With respect to sensitivity to bile salts and the protective effect of lecithin, CaCo-2 cells behaved very similar to erythrocytes. However, cholesterol failed to have any cytoprotective effect when used in combination with taurocholate or phosphatidylcholine/taurocholate solutions. Interestingly, at relatively high concentrations of cholesterol (cholesterol saturation index >1.0), the sterol even increased cytotoxicity. This correlated with a cholesterol-induced shift of phosphatidylcholine from micelles to vesicles, which normally occurs in supersaturated model and human bile. The different sensitivity of the two cell types to the effect of cholesterol on bile salt damage might be caused by the difference in lipid membrane composition. In conclusion, CaCo-2 cells represent a physiologically more relevant model system to study bile cytotoxicity than erythrocytes. When extrapolated to gallbladder epithelial cells, these results could be relevant for the pathogenesis of gallstone disease. The increased cytotoxicity might be the signal by means of which supersaturated bile induces mucin hypersecretion by gallbladder epithelial cells.

Bile salts (BS) are amphiphilic compounds that act as detergents when the concentration exceeds the critical micellar concentration (1). Therefore, it is not surprising that these compounds are cytotoxic. The cytolytic effect of BS has been shown for many different cell types, including hepatocytes (2), intestinal (3) and gallbladder (4,5) mucosal cells, and erythrocytes (6–8). However, studies on the mechanism of BS-induced cytolysis have been performed almost exclusively with erythrocytes (7–9). In pioneering studies Coleman (1) showed that cytotoxicity strongly depends on BS hydrophobicity. The cytolytic effect occurs exclusively at concentrations above the critical micellar concentration, the more hydrophobic BS, such as chenodeoxycholate and deoxycholate, being much more cytotoxic than the hydrophilic BS, such as cholate and ursodeoxycholate. The latter BS is almost nontoxic. In addition to the hydrophilic-hydrophobic balance of BS, cell membrane composition may also play a major role in the cytolytic effect (7,8,10). Particularly, the sphingomyelin content of

Abbreviations used in this paper: BS, bile salt(s); CF, carboxyfluorescein; CFDA, carboxyfluorescein diacetate; CH, cholesterol; CSI, cholesterol saturation index; DMEM, Dulbecco's modified Eagle's minimum essential medium; PC, phosphatidylcholine; TC, taurocholate; TCDC, taurochenodeoxycholate; TDC, taurodeoxycholate; TUDC, tauroursodeoxycholate.

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the membrane seems to be important. Sheep erythrocytes, which contain a relatively high amount of this lipid, were more resistant to BS than rat or guinea pig erythrocytes, which contain a relatively low amount of sphingomyelin (8). The role of other glycolipids and other membrane components has not yet been elucidated. The apical membrane of epithelial cells has been shown to contain relatively high amounts of glycolipids (11). Nevertheless, it has been demonstrated that these cells too are susceptible to BS-induced damage; e.g., solutions of pure BS cause histological damage to guinea pig gallbladder mucosa (4) and rabbit colonic mucosa (3). Therefore, the question arises how are the epithelia, which normally contact BS micelles, protected against these detergents? An important role could be played by biliary phospholipid. Coleman et al. (7) have shown that lecithin greatly reduces the cytotoxic effect of glycodeoxycholate on erythrocytes. The modulating effect of lecithin has not yet been tested systematically for other cell types; neither has the effect of the other important biliary lipid, cholesterol (CH), been investigated.

In an attempt to shed more light on the modulating role of phosphatidylcholine (PC) and CH in BS toxicity, we studied their influence on BS-induced cell damage in both human erythrocytes and a model system for epithelial cells. For this purpose we developed a cytoxicity assay for CaCo-2 cells, which are derived from a human colon adenocarcinoma cell line. These cells can be cultured in a polarized monolayer and have the morphological and biochemical characteristics of small intestinal absorptive cells (12,13). Therefore, they provide an excellent model system to study the sensitivity of the apical side of the cell to biliary solutions.

Materials and Methods

Materials

Taurine conjugates of cholate (TC), chenodeoxycholate (TCDC), deoxycholate (TDC), egg-yolk PC, and CH were purchased from Sigma Chemical Co. (St. Louis, MO); tauroursodeoxycholate (TUDC) and carboxyfluorescein diacetate (CFDA) were obtained from Calbiochem (La Jolla, CA). Bile salt and CFDA were in the highest purity available and were used in this study without further purification. Dulbecco's modified Eagle's minimum essential medium (DMEM) was obtained from Flow Laboratories (Irvine, G.B.). Other chemicals were of analytical grade. CaCo-2 cells were kindly donated by Professor J. M. Tager (Biochemistry Department, University of Amsterdam, The Netherlands); human blood samples were obtained from healthy volunteers by venipuncture and collected in tubes containing ethylenediaminetetraacetic acid (EDTA).

Preparation of Model Bile Solutions

Lipid mixtures were prepared as follows. Cholesterol was dissolved in chloroform and mixed with PC in chloroform/methanol (10:1). The organic solvents were evaporated under a stream of nitrogen at room temperature. The dry lipid film was then solubilized in BS solution, flushed with nitrogen, and incubated overnight at 80°C. Just before use, the samples were cooled to 37°C and filtered through a 0.22-μm filter. Table 1 summarizes the composition and the final concentration of each compound in the solutions used in the various experiments. Bile salts were dissolved in phosphate buffer (9.3 mmol/L Na_2HPO_4/1.3 mmol/L NaH_2PO_4, pH 7.4) or in HEPES buffer (15 mmol/L HEPES, pH 7.4), both solutions containing NaCl in appropriate amounts to maintain a constant ionic strength of 150 mmol/L.

Preparation of Cells

Human red blood cells were isolated and washed as described by Coleman et al. (7). Suspensions of fresh erythrocytes with a final hematocrit of 5% were used for experiments. CaCo-2 cells were cultured as described by Mohrmann et al. (15) with slight modifications. Briefly, CaCo-2 cells were grown in T-75 plastic flasks in DMEM supplemented with 20% fetal calf serum (Gibco, New England, NY), 50 U/mL penicillin, and 50 U/mL streptomycin (Flow Laboratories). Before confluency, the cells were split (split ratio, 1:8) as follows. CaCo-2 cells were rinsed twice with Hank's balanced salt solution (Gibco) and incubated for 5 minutes at 37°C with 1 mL of dissociation solution (Sigma), after which medium supplemented with 20% fetal calf serum was added to the cell suspension.

Table 1. Lipid Composition of Model Biles Used in the Experiments

Solutions	TLC (*g/dL*)	TC (*mmol/L*)	PC (*mmol/L*)	CH (*mmol/L*)	PC/TC	CSI (%)
a	3.38	50	12	—	0.24	—
b	4.31	50	24	—	0.48	—
c	6.17	50	48	—	0.96	—
d1	2.46	50	—	0.25	—	—
d2	2.47	50	—	0.50	—	—
d3	2.48	50	—	0.75	—	—
d4	2.49	50	—	1.00	—	—
a1	3.46	50	12	2.00	0.24	61
a2	3.51	50	12	3.40	0.24	101
a3	3.54	50	12	4.00	0.24	117
a4	3.66	50	12	7.00	0.24	194
a5	3.69	50	12	8.00	0.24	219
b1	4.39	50	24	2.00	0.48	37
b2	4.44	50	24	3.40	0.48	61
b3	4.47	50	24	4.00	0.48	71
b4	4.58	50	24	7.00	0.48	119
b5	4.62	50	24	8.00	0.48	135
b6	4.70	50	24	10.00	0.48	164
b7	4.78	50	24	12.00	0.48	191
b8	4.85	50	24	14.00	0.48	218

TLC, total lipid concentration.

Monolayers were grown in microwell plates in supplemented DMEM, which was replaced with fresh medium every 2 days. After 12–14 days, the postconfluent cultures were used for experiments.

Cytoxicity Assay

Human red blood cells were suspended in HEPES buffer solution (15 mmol/L HEPES/154 mmol/L NaCl, pH 7.4) to a final hematocrit of 5%. To test the modulating activity of PC and CH, the erythrocyte suspension was incubated at 37°C for 15 minutes with the solutions shown in Table 1. After incubation, the samples were centrifuged for 1 minute at 10,000 × *g*, and the extent of lysis was assayed in the supernatant. The absorbance values of appropriate dilutions of the samples were read at 540 nm and compared with that of erythrocytes lysed in distilled water. CaCo-2 cell were loaded with the hydrophilic fluorophore 6-carboxyfluorescein (CF) by means of its membrane-permeable diacetate ester, 6-CFDA (16), as follows. Monolayers of CaCo-2 cells plated in microwell plates were washed two times with phosphate buffered saline (PBS). The cells were then incubated with 50 μmol/L of CFDA for 15 minutes at 37°C. Loading of the cells was optimal under these conditions. At the end of the incubation, the medium was removed and the cells were washed three times with PBS to eliminate any residual CFDA. Bile salt cytotoxicity was determined by incubating the cells for 5 minutes at 37°C with the solutions described above. At the end of incubation, the medium was collected and the cells were treated with 0.1% Triton X-100. The CF content of both incubation medium and Triton X-100 solution was read fluorimetrically (excitation, 490 nm; emission, 516 nm) using a Perkin–Elmer 3000 fluorimeter (Gouda, Netherlands). Membrane damage was expressed as percentage of leakage of CF from the cells. All determinations were performed in quadruplicate.

Gel Filtration

Model bile mixtures were subjected to gel filtration according to the method described by Somjen and Gilat (17). Chromatographic analysis was performed on Sephacryl S-300 (Pharmacia, Uppsala, Sweden) columns (16 × 400 mm) with a flow rate of 0.5 mL/min. The elution buffer was PBS supplemented with 10 mmol/L sodium cholate.

Chemical Analysis

Protein was determined according to the method of Lowry et al. (18). Membrane lipids were extracted using the procedure of Folch et al. (19). Phospholipid was determined by the choline oxidase method, which is specific for PC (20). Cholesterol was measured enzymatically (21).

Statistical Analysis

Student's paired *t* test or the one-way analysis of variance (ANOVA) test were used to compare cytotoxicity of different solutions. Values are expressed as mean ± SE. Statistical comparison between the degree of cytotoxicity and the lipidic distribution of the micellar phase was evaluated by Spearman's test.

Results

The Effect of Phosphatidylcholine and Cholesterol on Taurocholate-Induced Hemolysis

When erythrocytes are incubated with 50 mmol/L TC for 15 minutes, cytolysis amounts to about 95% (Figure 1*A*). Phosphatidylcholine reduces the hemolytic effect profoundly. Already at a PC-BS ratio of 0.2 cytolysis decreases significantly. All BS-induced cytotoxicity is abolished at a PC-BS ratio of 0.8. Surprisingly, CH is at least 10 times more effective in reducing the cytolytic effect (Figure 1*B*). Even

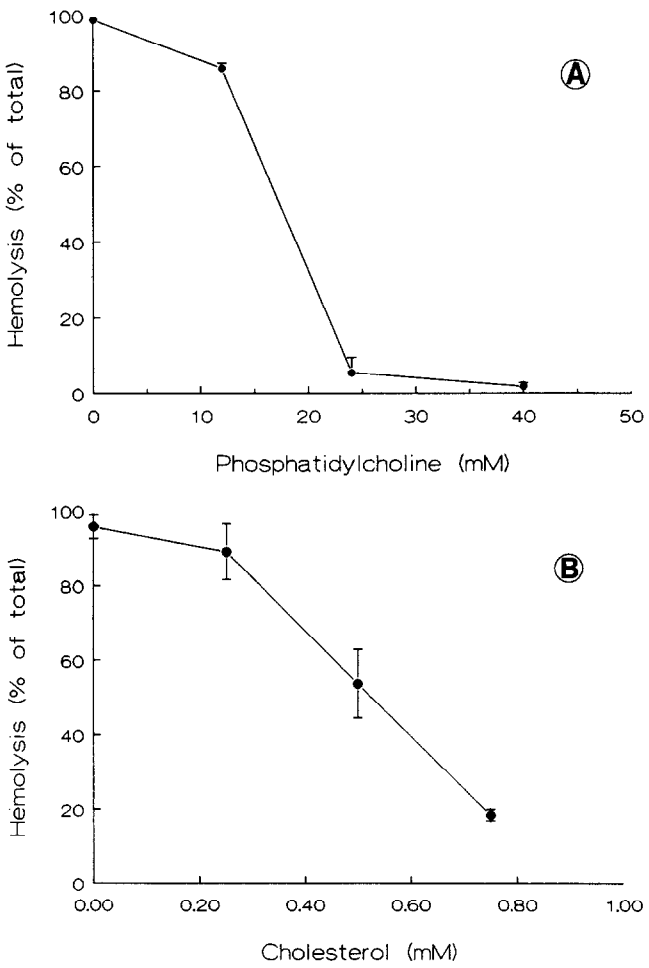

Figure 1. Effect of PC and CH on BS-induced lysis of human erythrocytes. Red blood cells were incubated for 15 minutes at 37°C with 50 mmol/L TC in the presence or absence of different amounts of (*A*) PC and (*B*) CH. Hemolysis was measured by release of hemoglobin. Values are expressed as mean ± SE for three different experiments.

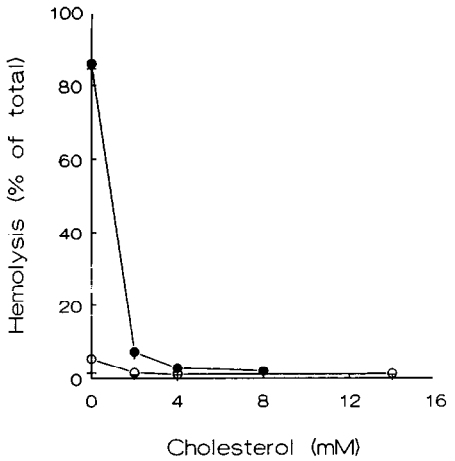

Figure 2. The hemolytic effect of model bile. Red blood cells were incubated for 15 minutes at 37°C with PC/TC solutions (●, 12 mmol/L PC/50 mmol/L TC; ratio, 0.24: ○, 24 mmol/L PC/50 mmol/L TC; ratio, 0.48) supplemented with varying amounts of CH as indicated in the figure. Values are given as mean ± SE for three different experiments.

at a CH-BS ratio of 0.01, cytolysis decreases about 40%. Cytoxicity is abolished almost completely at a CH-BS ratio of 0.015. To investigate whether cholesterol has an additional effect on the PC-induced decrease in cytotoxicity, we have performed the experiments shown in Figure 2. Erythrocytes were incubated with either 12 mmol/L PC/50 mmol/L TC or 24 mmol/L PC/50 mmol/L TC and varying amounts of CH. It is clear that CH enhances the protective effect of PC. Addition of 2 mmol/L CH abolishes all cytoxicity. The profound protective effect of CH cannot be simply caused by formation of CH/BS mixed micelles, because of the relatively low affinity of simple BS micelles for CH (22). Therefore, we investigated whether CH could protect erythrocytes against BS damage by a direct interaction with the plasma membrane of red blood cells. Table 2 shows that after incubating erythrocytes with a CH/BS solution (75 μmol/L CH/30 mmol/L TC) the lipidic content of the membrane had changed. There was a marked increase in CH content and a concomitant reduction in PC content. The consequence of this lipidic change is a strong increase in CH-PC ratio of the membrane of erythrocytes.

Table 2. Effect of Incubation With Cholesterol/Taurocholate Solution on the Membrane Composition of Erythrocytes

Erythrocytes	PC (*μmol/g protein*)	CH (*μmol/g protein*)	CH/PC
Control	4.79	9.07	1.89
Treated	2.63	18.70	7.11

NOTE. Erythrocyte suspensions (hematocrit 5%) were incubated in four volumes of HEPES/NaCl (15/150 mmol/L, pH 7.4) for 15 minutes at 37°C with or without 75 μmol/L CH/30 mmol/L TC. Values are the mean of two determinations.

Effect of Bile Salts on Membrane Integrity of CaCo-2 Cells

Because the membrane composition of erythrocytes (23) is very different from that of epithelial cells, we investigated whether this influences the dynamics of model bile cytotoxicity on cultured CaCo-2 cells, a cell-line derived from a colon carcinoma (12). These cells form a polarized monolayer in culture and, therefore, can serve as a model system for gastrointestinal epithelium (13). To monitor the cytolytic effect of model bile solutions, we developed a rapid and simple assay. CaCo-2 cells were incubated with CFD. This compound is permeable but once it is inside the cells, it is hydrolyzed by cytosolic esterases to form the impermeable fluorescent organic anion CF (16). Membrane damage can be monitored simply by measuring CF release from the cells. Figure 3 depicts the kinetics of CF release induced by TC and TDC. Release of the fluorescent dye shows a rapid initial phase that plateaus at about 4 minutes. A small amount of CF leaks from the cells also in the absence of BS. Because a quasisteady state is apparent after 5 minutes, we used this incubation period in the experiments described below. All results were corrected for CF release in the absence of BS. Figure 4 shows the cytotoxic potency on CaCo-2 cells of four different taurine-conjugated BS (TC, TDC, TCDC, and TUDC). No lysis was observed at concentrations below the CMC for the different BS. Above the critical micellar concentration (CMC) release of CF increases sharply. However, the slope of the increases varies markedly for the different BS. The slope for the dihydroxy BS

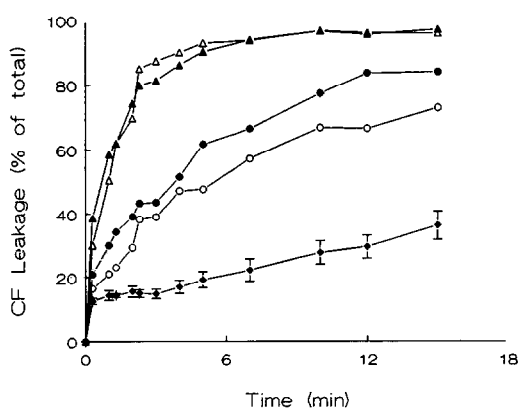

Figure 3. Kinetics of BS-induced release of CF from CaCo-2 cells. CaCo-2 cells were loaded with the fluorescent compound CF as described in Materials and Methods. Membrane damage was monitored by measuring CF leakage from cells after incubation with PBS (◆) or PBS containing TDC (●, 2 mmol/L; ▲, 5 mmol/L) or TC (○, 20 mmol/L; △, 50 mmol/L) for the time interval indicated.

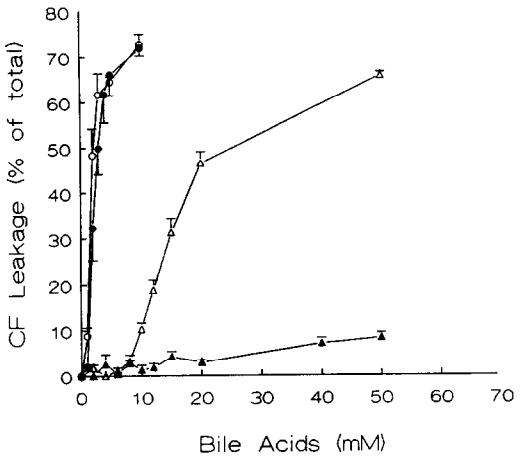

Figure 4. The sensitivity of CaCo-2 cells to different BS. Carboxyfluorescein-loaded CaCo-2 cells were incubated for 5 minutes at 37°C with TDC (○), TCDC (●), TC (△), and TUDC (◆). Data were corrected for CF released in a control incubation with PBS. Values are expressed as mean ± SE for three different experiments.

TDC and TCDC is very steep, and the maximum lytic effect for these BS is obtained at 10 mmol/L. Similar concentrations of TC are much less toxic, whereas almost no toxicity is observed for TUDC.

Effect of Phosphatidylcholine and Cholesterol on Taurocholate-Induced Lysis of CaCo-2 Cells

The effect of PC on TC-induced leakage of CF from CaCo-2 cells was similar to that observed in the erythrocyte model. At low concentrations of the phospholipid, the effect was somewhat stronger but also in the case of CaCo-2 cells an optimal effect was reached at 40 mmol/L PC (Figure 5*A*). In contrast, the effect of CH was entirely different. As shown in Figure 5*B* at all concentrations used, CH failed to have any effect on CF leakage from the CaCo-2 cells. A possible additional effect of CH on PC-decreased cytolysis was studied as described for the erythrocyte system. The results are given in Figure 6. At the lower range of CH concentrations (CSI $\leq$ 1), the sterol has no effect on CF leakage. However, when the cholesterol saturation index (CSI) increased above 1, there was a small but highly significant increase in cytotoxicity. Since CH apparently has no direct effect on CaCo-2 cell integrity, the latter effect must have been mediated through the PC effect.

It has been shown in a number of studies that in supersaturated model bile the surplus of CH is carried by CH/phospholipid vesicles (17,24–26). To be able to solubilize all the extra CH, a shift of phospholipid must have occurred from the micellar to the vesicular phase. To verify this assumption, we have separated the micellar and vesicular phase in the model bile mixtures by using Sephacryl-S300 column chromatography as has been described by Somjen and Gilat (17). The results are given in Table 3. It is clear that indeed an increase in CH shifts lecithin from the micellar to the vesicular phase. There is a clear correlation between the amount of lecithin in the micellar phase and the increase in cytoxicity (Figure 7).

Discussion

The damaging effect of BS on cellular membranes is a well-established phenomenon. It has been implicated to play an important role in colon tumorigenesis (27–29). When BS can have such profound effects at the relatively low concentrations prevailing in the colon, one wonders how the epithelial cells in the biliary tree are protected against these potent detergents. Wingate et al. (30) showed that addition of PC to BS solutions greatly decreased their effect on

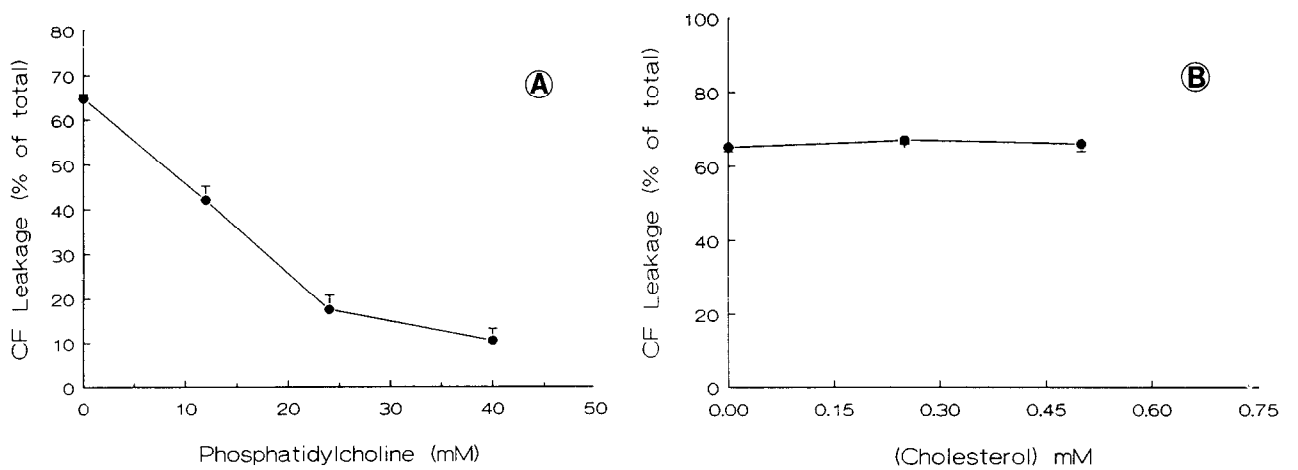

Figure 5. The effect of PC and CH on BS-induced CF leakage from CaCo-2 cells. Phosphate-buffered saline containing TC (50 mmol/L) was supplemented with (*A*) PC or (*B*) CH at the concentrations indicated in the figure. Membrane damage was monitored by determining CF leakage after 5 minutes of incubation at 37°C. Data were corrected for CF leakage in the control incubation.

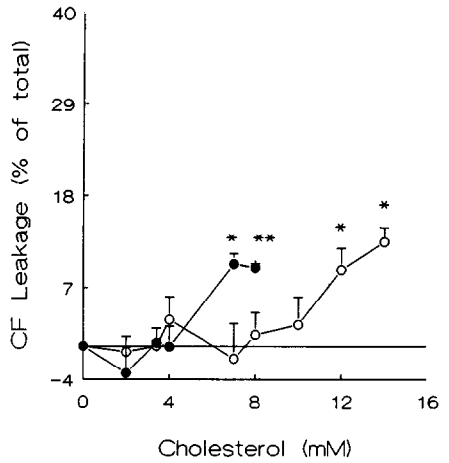

Figure 6. Cytolytic effect of model bile on CaCo-2 cells. Carboxyfluorescein-loaded CaCo-2 cells were incubated with PC/TC solutions (●, 12 mmol/L PC/50 mmol/L TC; ratio, 0.24: ○, 24 mmol/L PC/50 mmol/L TC; ratio, 0.48) supplemented with varying amounts of CH as indicated in the figure. To compare the two sets of data directly, CF leakage was corrected for the amounts released from the cells in the absence of CH. Carboxyfluorescein leakage in the presence of 12 mmol/L PC/50 mmol/L TC was 35.6% ± 5.3%; with 24 mmol/L PC/50 mmol/L TC, CF release was 18.7% ± 2.8%. Data are given as mean ± SE for three different experiments. *$P < 0.05$; **$P < 0.01$.

glucose and water absorption in jejunum. More direct evidence for a protective effect of PC was presented by Coleman (1). Addition of lecithin to either bile or glycodeoxycholate decreased hemolysis of human erythrocytes dramatically. In his view, PC could prevent micellar extraction of phospholipid from cellular membranes through the formation of BS/PC mixed micelles. Coleman did not investigate the effect of CH. In the present study we have shown that the protective effect of CH on BS-induced hemolysis is much stronger than that for lecithin. Even at a CH-BS ratio of 0.015 CH abolishes almost all hemolysis induced by TC. Simple BS micelles have a relatively low affinity for CH (22). Therefore, it is unlikely that formation of mixed micelles plays a role in the protective effect of CH. More probably, CH acts directly on the erythrocyte membrane. Indeed, as shown in Table 2, incubation of erythrocytes with 75 μmol/L CH/30 mmol/L TC increases the CH content of the erythrocyte membrane dramatically. Concomitantly, the PC content decreases. Probably the resulting high CH-PL ratio in the membrane protects the erythrocytes against attack by BS micelles. Presumably, the extra effect of CH on PC-induced inhibition of cytolysis can be explained by the same mechanism. However, it can not be excluded that in this case incorporation of CH in the mixed micelles prevents further extraction of CH from the erythrocyte membrane.

The gastrointestinal cells that are in contact with bile have not been reported to have an unusual high CH content in their membrane (11). To address the question whether the results obtained with erythrocytes can be extrapolated to epithelial cells, we have performed similar experiments with a cell line derived from a human colon carcinoma. These cells form a polarized monolayer in culture characterized by the presence of brush border microvilli only at their apical surface and by the formation of both tight junctions and desmosomes (12). Under our conditions, the bile acid solutions come into contact with only the apical side; therefore, it is likely that our findings apply to the apical side of the cells specifically (12,13). The susceptibility of CaCo-2 cells to BS-induced damage increased in the order TUDC < TC < TCDC < TDC. In this respect, the CaCo-2 cells did not behave differently from other cell types, including erythrocytes (1). As has also been found for

Table 3. Lipid Distribution Over the Micellar and Vesicular Phase in Model Bile

Model bile	Lipid in micelles				Lipid in vesicles			
	mmol/L		*%*		*mmol/L*		*%*	
	CH	PC	CH	PC	CH	PC	CH	PC
a1	1.8	10.2	100	100	—	—	—	—
a2	2.1	10.0	63.9	91.3	1.16	0.9	36.1	8.6
a3	1.95	9.8	59.1	91.1	1.35	0.9	40.9	8.8
a4	1.74	8.9	33.0	78.6	3.53	2.4	67.0	21.3
a5	1.86	7.5	29.8	74.9	0.38	2.5	70.2	25.1
b1	2.07	22.3	100	100	—	—	—	—
b2	3.48	21.4	100	100	—	—	—	—
b3	3.79	20.9	100	100	—	—	—	—
b4	3.68	20.1	73.0	95.6	1.36	0.9	27	4.4
b5	3.41	17.3	62.9	93.5	2.01	1.2	37.1	6.5
b6	2.94	17.1	37.1	82.9	4.99	3.5	62.9	17.1
b7	0.89	15.0	30.9	77.1	6.52	4.4	69.7	22.9
b8	2.78	13.8	26.5	72.0	7.72	5.3	73.5	28.0

NOTE. Model bile was chromatographed on Sephacryl S-300 as described in Materials and Methods section. The abbreviations used to indicate the different model bile solutions refer to Table 1. The composition of these model biles is given in Table 1.

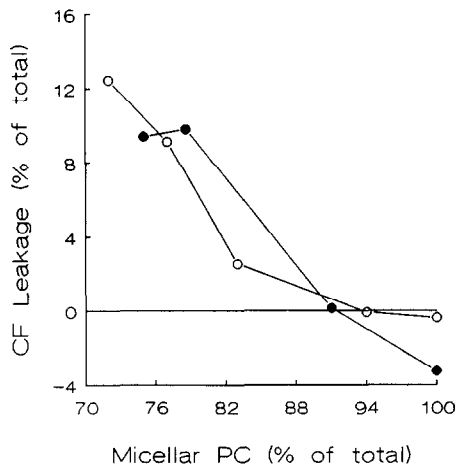

Figure 7. Correlation between the extent of CF leakage from CaCo-2 cells and the micellar content of PC in model bile. The amount of micellar PC in model bile (●, 12 mmol/L PC/50 mmol/L TC; ○, 24 mmol/L PC/50 mmol/L TC) containing various amounts of CH was determined by gel filtration on Sephacryl S-300 as described by Somjen and Gilat (17). The lipid concentration in the peak fractions for the different model biles is given in Table 3. Data for CF leakage induced by the different model bile solutions have been taken from Figure 6.

other cell types cytoxicity occurs only above the CMC of the different BS (1,6,9), indicating that also in the case of CaCo-2 cells only micelles are toxic. As has also been shown for other cell types, micelles from TDC and TCDC are much more toxic than micelles from TC or TUDC. In fact, under the experimental conditions used in this study TUDC induced almost no CF release. An explanation for the variation in cytotoxic potency of the different micelles might come from studies by Mazer et al. (31). By using quasielastic light scattering, they showed that at concentrations above the CMC, TDC and TCDC form relatively large aggregates, which these investigators called secondary micelles. Possibly these larger micelles have a larger capacity for membrane lipids. The lack of cytotoxicity by TUDC could be explained by the low affinity of this BS for PC (32).

In this study we have only investigated the effect of taurine conjugates of the BS. However, because the difference in the hydrophilic/hydrophobic balance between tauro- and glyco-conjugates is only slight (33), one can expect that the results will be similar for the glyco-conjugates. Indeed, in a preliminary study Guldutuna et al. (34) have shown that there was almost no difference in the membranolytic effect of tauro- and glyco-conjugates. With respect to the modulating effect of PC on TC-induced cytotoxicity, CaCo-2 cells behaved similar to erythrocytes. However, in contrast to erythrocytes, CH showed no protective effect on CaCo-2 cells. The CH content of the plasma membranes of both cell types is similar, i.e., 30%–40% in both cell types. However, there is a marked difference in the content of glycolipids. For human erythrocytes a glycolipid content of about 10 mol % has been reported (23), whereas the apical membrane of gastrointestinal epithelia contains about 37 mol % of glycolipid (11). Moreover, all glycolipids are localized in the outer leaflet of the lipid bilayer (11), so that the glycolipids are by far the most abundant membrane constituent with which the micelles come in contact. In a recent study, Thomas and Poznasky (35) have shown that glycolipid facilitates transfer of CH between membranes. Apparently, membranes with a high glycolipid content have a relatively low affinity for CH. The effect was not caused by increased membrane rigidity generated by the gangliosides, because sphingomyelin, which has the same hydrocarbon moiety, has an opposite effect on CH transfer (35). More probably, the sugar residues of the glycolipid are involved, although the exact interaction of these residues with CH is not yet clear. Because the lipid content of the CaCo-2 cells resembles much more the lipid content of the cells in the gastrointestinal tract, we feel that the conclusion that CaCo-2 cells represent a more relevant model system to study bile cytotoxicity than erythrocytes seems justified.

It has frequently been demonstrated that supersaturation of bile with CH induces mucin hypersecretion in the gallbladder (36,37). No explanation for this phenomenon is as yet available. Because CH alone apparently has no direct protective effect on CaCo-2 cells, we postulated that high concentrations of the sterol could lead to an increased cytotoxicity. This postulate was based on the new insights in the dynamics of CH solubilization presented in the last few years. It has now become clear that to solubilize "excess" CH in bile, phospholipid has to shift from the micellar phase to the vesicular phase in bile (17,24–26). This shift will lead to an increase in BS micelles nonsaturated with PC, which should in turn increase cytotoxicity. As shown in Figure 6 this was indeed observed. Under a CSI of 1, CH had no effect, but at higher CSI levels a small but significant increase in cytotoxicity was observed. Because vesicles per se have no cytolytic effect (not shown), this increased cytotoxic effect must have been caused by an increased micellar toxicity. To substantiate this point, we have determined the amount of lecithin solubilized in the micellar phase by using gel permeation chromatography. Figure 7 shows that there is indeed a strong correlation between cytotoxicity and the amount of PC in the micelles. In conclusion, our results show that both phospholipid and CH modulate BS-induced cytotoxicity, the effect of CH being dependent on the cell type used. When extrapolated to gallbladder epithelial cells, these results could be relevant for the pathogenesis of gallstone disease. The increased cytotoxicity might be the signal via which

supersaturated bile induces mucin hypersecretion by gallbladder epithelial cells.

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