REVISTA ESPAÑOLA DE FISIOLOGIA, 39, 69-76. 1983

Influence of Bile Salts on the Endogenous Excretion of Bile Pigments

J. González, F. Hidalgo, M. A. López * and A. Esteller **

Departamento Interfacultativo
de Fisiología Animal
Universidad de Granada
Granada (Spain)

(Received on June 30, 1982)

J. GONZALEZ, F. HIDALGO, M. A. LOPEZ and A. ESTELLER. *Influence of Bile Salts on the Endogenous Excretion of Bile Pigments.* Rev. esp. Fisiol., 39, 69-76. 1983.

Effect of the infusion of glycodeoxycholate (GDC), taurocholate (TC) and dehydrocholate (DHC) on bile flow and on bile salt, biliary lipid and bile pigment secretion, has been studied in pentobarbital-anesthetized rabbits. GDC increased bile flow the most, while DHC increased it more than TC. The different choleretic actions of these bile salts cannot be explained by means of variations in their capacity to form micelles. Only GDC and TC were able to stimulate biliary lipid secretion, which suggests that both bile salts increase the formation of mixed micelles. GDC and TC to a lesser extent increased bile pigment excretion, DHC being without effect. These results favour the hypothesis that micellar binding could be an important factor responsible for the effect of bile acids on bile pigment excretion and should not be completely ruled out.

The liver is the organ in which the uptake, conjugation and excretion of bile pigments takes place. These processes are closely related to those belonging to canalicular bile secretion in which bile salts plays a major role, so that not only the flow of bile may be altered but also its composition. Thus, it is known that, to a greater or lesser extent, the different bile salts increase in some species choleresis (18, 24, 26), the transport maximum of bilirubin (12) or bromosulfthalein (2) and the excretion of cholesterol and phospholipids (15).

The results of works using micelle-forming and non micelle-forming bile salts suggest that the capacity for micelle aggregation could be an important factor in the maximal biliary excretion of numerous organic anions (25), among them the bile pigment, there being at times an apparent relationship between the capacity of the bile acids to form micelles and the excretion of such anions. Sup-

Present address:

* Departamento de Fisiología Animal. Facultad de Biología. Salamanca (Spain).

** Departamento de Fisiología Animal. Facultad de Farmacia. Salamanca (Spain).

porting this explanation is the fact that in man dehydrocholate, a non micelle-forming bile salt, decreases the biliary excretion of bilirubin and bromosulfthalein (3). However, dehydrocholate increases the maximal excretion of dibromosulfthalein in the rat more than taurocholate in spite of the greater capacity of the latter for micelle aggregation (28), and glycodihydrofusidate, a micelle forming steroid, has no effect on the maximal excretion of different anions (5). In any case, information corcening the endogenous excretion of bile pigment and the action of different bile acids on it is still scarce and often confusing (6).

In order to clarify the role of bile salts, with or without the capacity to form micelles, in the endogenous excretion of biliverdin and bilirubin, a study has been carried out of the effect caused by the infusion of glycodeoxycholate (GDC), taurocholate (TC) and dehydrocholate (DHC) on both the flow and composition of bile in anesthetized rabbits, GDC and TC were included as physiological micelle-forming bile salts, the first being predominant in rabbit bile; DHC was chosen as a synthetic bile salts which *in vitro* do not form micelles.

Materials and Methods

Experimental Protocols. Castilian male and female rabbits (weight range 1.7-2.3 kg) were used after they had fasted for 24 h with free access to water. The animals were anesthetized with sodium pentobarbital (Nembutal, 25 mg/kg body wt) injected into an ear vein. Body temperature was maintained constant at $39 \pm 0.5°$ centigrade.

The animals were tracheotomized and a catheter (B, Braum Melsungen n.° 2) was inserted in the right femoral vein for the administration of fluids by means of a calibrated peristaltic pump. After tying off the cystic duct and the pylorus, the choledocus was cannulated with a polyethylene catheter. Bile samples were collected at 20 min intervals in previously weighed tubes which were wrapped in aluminium foil to avoid the photooxidation of the bile pigments.

After an equilibrium period of 1 h, two samples were collected without infusion, followed by the infusion of bile salts for periods of 40 min at increasing rates of 0.25, 0.50, 0.75 and 1.00 mg $\times$ $min^{-1} \times kg^{-1}$. One control group did not receive infusion. The bile salts infused were sodium glycodeoxycholate, taurocholate and dehydrocholate (Sigma) dissolved in 0.9 % NaCl and 0.1 M phosphate buffer (pH = 7.5) with the addition of 3 % bovine albumin (Sigma) in order to avoid the haemolytic effects of the bile salts (24).

Analytical methods. The following determinations were carried out in bile: sodium and potassium concentrations by flame photometry; chloride by potentiometric volumetry; biliverdin according to the method of LARSON *et al.* (19); bilirubin according to the method of MALLOY and EVELYN (22); total lipids by the use of a vainillin- phosphoric reagent (16), after obtaining the biliary lipid extract (11).

In the rabbit, sodium glycodeoxycholate makes up about 80-90 % of the bile salts present in bile (10, 13), so that in all cases deoxycholate was measured (20), its values being considered as equivalent to those of total bile salts. When taurocholate or dehydrocholate were infused they were also measured specifically according to the methods of IRVIN *et al.* (17) and BARTOS (1), respectively.

Results were expressed as mean values $\pm$ standard error of the mean (SEM). Wilcoxon's test was used for the determination of statistically significant differences. Regression lines were calculated by the least squares method.

Results

In the control group, bile flow decreased in the region of 30 % throughout the experiments and there was also a decrease in the concentration of bile salts (table I). This fall in bile flow is similar to that described by ESTELLER *et al.* in anesthetized (8) and conscious (9, 10) rabbits and may be attributed to an interruption of the enterohepatic circulation of bile salts.

Within the range of infusion rates studied by us, GDC increased bile flow to a greater extent than DHC, and both of these more than TC (table I). Figure 1 shows the choleretic efficiency which for GDC was 31 μl/μEq of infused bile salt; for DHC 22 μl/μEq and for Tc 13 μl/μEq.

With respect to the electrolytes studied, sodium and potassium concentrations showed a tendency to increase compared to the control group in GDC and TC infusions (table I). Chloride concentration

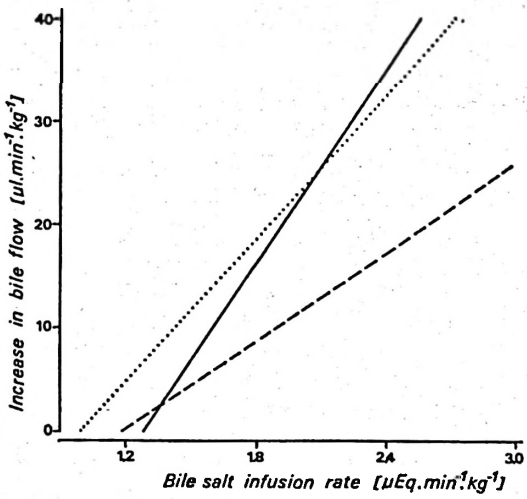

Fig. 1. *Increases in bile flow above control levels induced by the infusion of bile salts.* Regression lines are shown for glycodeoxycholate (——) (y = 31x — 40; r = 0.899), taurocholate (-----) (y = 13x — 15; r = 0.876) and dehydrocholate (.....) (y = 22x — 19; r = 0.884).

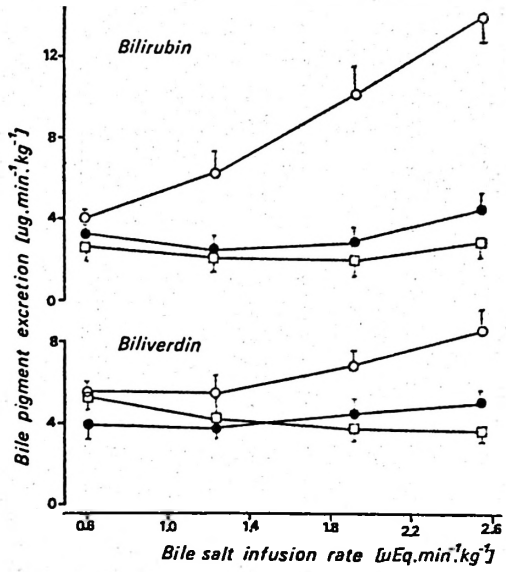

Fig. 2. *Effect of glycodeoxycholate (○), taurocholate (●) and dehydrocholate (□) infusion on biliary excretion of biliverdin and bilirubin.*
Mean values ± SEM.

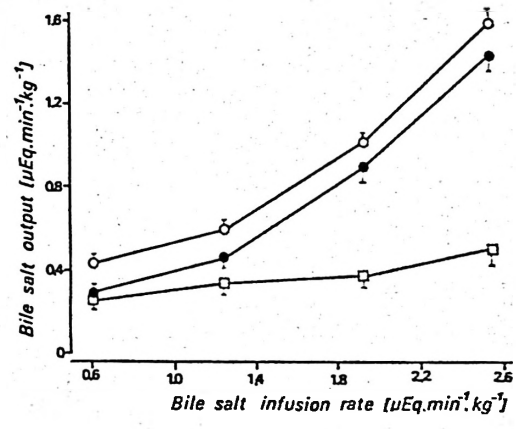

Fig. 3. *Effect of the infusion of glycodeoxycholate on the secretion rate of deoxycholate (○), of the infusion of taurocholate on the secretion rate of deoxycholate + cholate (●), and of the infusion of dehydrocholate on the secretion rate of deoxycholate + dehydrocholate (□).*
Mean values ± SEM.

Table I. *Effect of infusion of bile salts on bile flow and concentration of biliverdin, bilirubin, total lipids, bile salts, sodium, potassium and chloride in bile.*

Each value is mean ± SEM of five animals. Assay: CO (control), (GDC) (glycodeoxycholate infusion), TC (taurocholate infusion) and DHC (dehydrocholate infusion). Periods: A (without infusion), B (infusion at a rate of 0.25 mg × min^{-1} × kg^{-1}), C (infusion at a rate of 0.50 mg × min^{-1} × kg^{-1}), D (infusion at a rate of 0.75 mg × min^{-1} × kg^{-1}) and E (infusion at a rate of 1.00 mg × min^{-1} × kg^{-1}). In TC and DHC assays, bile salt concentration refers to concentration of deoxycholate + cholate and deoxycholate + dehydrocholate respectively; in CO and GDC assays refers to concentration of deoxycholate only.

Period 40 min	Flow $\mu l/min^{-1}/kg^{-1}$	Biliverdin mg/100 ml^{-1}	Bilirubin mg/100 ml^{-1}	Total lipid mg/100 ml^{-1}	Bile salt mEq/l^{-1}	Sodium mEq/l^{-1}	Potassium mEq/l^{-1}	Chloride mEq/l^{-1}
Control (CO)								
A	82 ± 8	6.3 ± 1.2	5.6 ± 0.9	29 ± 2	4.8 ± 0.6	150 ± 3	3.3 ± 0.1	87 ± 3
A	73 ± 6	5.8 ± 1.3	4.6 ± 0.7	30 ± 2	4.3 ± 0.8	155 ± 5	3.2 ± 0.4	89 ± 4
A	69 ± 6	5.4 ± 1.4	3.5 ± 0.5	33 ± 2	4.2 ± 0.7	153 ± 5	3.5 ± 0.3	95 ± 3
A	59 ± 6	4.8 ± 1.2	3.4 ± 0.4	30 ± 2	3.9 ± 0.6	155 ± 6	3.6 ± 0.4	91 ± 3
A	57 ± 6	5.3 ± 1.5	3.5 ± 0.6	28 ± 3	3.8 ± 0.5	149 ± 7	3.3 ± 0.3	93 ± 4
Glycodeoxycholate (GDC)								
A	102 ± 9	7.3 ± 1.3	5.5 ± 0.5	25 ± 2	4.4 ± 0.3	136 ± 9	3.0 ± 0.2	86 ± 5
B	93 ± 5	6.0 ± 0.7	4.3 ± 0.4	30 ± 2	5.2 ± 0.5	160 ± 6	3.7 ± 0.1	97 ± 3
C	94 ± 4	6.2 ± 1.3	6.9 ± 1.3	38 ± 7	7.0 ± 0.5	160 ± 9	3.6 ± 0.2	93 ± 1
D	107 ± 6	6.2 ± 0.7	9.4 ± 2.0	43 ± 7	9.3 ± 0.6	162 ± 9	3.9 ± 0.2	91 ± 3
E	133 ± 4	6.0 ± 1.0	11.0 ± 1.5	47 ± 6	12.0 ± 0.8	168 ± 7	4.2 ± 0.1	87 ± 7
Taurocholate (TC)								
A	101 ± 15	4.9 ± 1.3	3.1 ± 0.2	20 ± 2	3.6 ± 0.3	139 ± 11	3.5 ± 0.4	87 ± 8
B	97 ± 13	4.1 ± 0.9	3.3 ± 0.5	23 ± 2	3.9 ± 0.5	147 ± 7	3.6 ± 0.4	97 ± 4
C	99 ± 10	4.6 ± 1.1	2.8 ± 0.6	27 ± 2	5.0 ± 0.4	152 ± 7	3.7 ± 0.4	94 ± 9
D	107 ± 12	3.9 ± 0.8	2.7 ± 0.5	26 ± 2	8.8 ± 1.4	158 ± 6	3.9 ± 0.4	92 ± 4
E	116 ± 12	4.0 ± 0.7	3.6 ± 1.0	32 ± 2	13.3 ± 2.3	163 ± 5	4.0 ± 0.4	91 ± 2
Dehydrocholate (DHC)								
A	83 ± 12	8.4 ± 1.5	4.3 ± 1.6	27 ± 4	4.0 ± 0.3	149 ± 6	2.9 ± 0.3	83 ± 3
B	92 ± 11	5.8 ± 1.0	3.0 ± 1.2	24 ± 3	3.4 ± 0.2	148 ± 6	2.8 ± 0.1	81 ± 3
C	101 ± 7	4.1 ± 0.7	2.6 ± 1.0	24 ± 3	3.5 ± 0.2	155 ± 8	3.0 ± 0.1	80 ± 4
D	110 ± 4	3.2 ± 0.5	2.0 ± 0.7	23 ± 3	3.4 ± 0.2	153 ± 7	3.1 ± 0.3	78 ± 6
E	131 ± 5	2.5 ± 0.3	1.8 ± 0.7	20 ± 2	4.1 ± 0.3	148 ± 5	3.1 ± 0.4	72 ± 3

decreased in the case of DHC (table I). However in all cases output increased, specially with infusions of GDC.

Regarding the secretion of biliary lipids, concentration increased to a considerable extent both with infusions of GDC and TC, the opposite to the case of DHC (table I). Similar were the results for bile pigment concentration (table I) and, in consequence, in infusions with DHC there was no positive effect on the excretion of bile pigments as in the case of the other two bile salts.

Discussion

Choleretic efficiency of the bile salts. Several authors have attempted to explain the different chaleretic action of the various bile salts as a function of variations in their physico-chemical properties, fundamentally the capacity to form micelles dependent on structure and polarity (4). It is clear from our results (fig. 1) that the choleretic actions of GDC and DHC are similar and greater than that induced by TC, coinciding in this sense with the data presented in the literature (7, 18, 24, 26). This phenomenon may not be explained on the basis of the capacity for micelle formation, which according to several authors (14, 27) and our own data on biliary lipids (table I) is greater for GDC than for TC and null for DHC, since in such circumstances choleresis should be greater for DHC and lesser for GDC and TC. As a consequence, unless these bile salts have unusual physico-chemical properties in rabbits bile (24), it is difficult to explain the difference in their choleretic action.

Recent findings by O'MAILLE (23) seem to indicate that the bile salts might act on the mechanisms of bile formation independent of bile salts, in particular on the active secretion of sodium; the same idea is supported by the study of LUPIANI *et al.* (21). The action must be more important in the case of GDC, since choleresis should invert the negative action of micelle formation.

Metabolization of dehydrocholate. If the secretion rate of bile salts in bile is analyzed when each bile salt is infused at increasing doses (fig. 3), it may be seen that the behaviour of GDC is very close to that of TC, with similar recoveries. However, DHC is recovered in much smaller amounts; since the determination of DHC was carried out by a method which is specific for 3-keto steroids, it is higly possible, and here our results coincide with those of literature (6, 27) that before its biliary secretion DHC might have undergone modifications by the cells of the hepatic parenchyme (probably a reduction of the keto group at position three), giving rise to different metabolites.

Secretion of biliary lipids. Our results show that only GDC and TC were able to increase biliary lipid concentration (table I), which would reflect the formation of mixed micelles. On the other hand, the infusion of DHC decreased lipid concentration and it may be concluded that under our experimental conditions, neither DHC nor its metabolites form micelles, though this does not necessarily exclude the possibility of micelle formation if we are below the critical micelle concentration for DHC metabolites.

Excretion of bile pigments. Of the three bile salts analyzed by us only GDC and TC increased the excretion of bile pigments, specially bilirubin (fig. 2). These results seem to support the theory of SCHARSCHMIDT and SCHMID (25) which postulates the existence of some kind of union of the bile pigments to the bile salt micelles, facilitating their transport and biliary excretion. Thus, GDC with the maximal capacity for micelle aggregation gives rise to a very pronounced increase in the concentration and excretion of bil-

irubin in bile, together with, though at lesser extent, an increase in biliverdin excretion. TC, with an intermediate capacity for micelle aggregation, causes less effect, though still considerable when compared with the drop in the excretion of pigments in the absence of infusion. Finally, DHC and its metabolites, in capable in our case of forming micelles, do not favour in any way the excretion of bile pigments. Nevertheless we must point out that the micelle factor is not probably the only one and, according to the data reported by other authors, there exist the possibility of specific interactions at the transport level (2, 28).

Apart from what has been described above, our results also permit the postulation of a possible stimulatory action of the bile salts on the step from biliverdin to bilirubin, which is very clear in the case of GDC.

Resumen

Se estudia el efecto de la infusión de glicodeoxicolato (GDC), taurocolato (TC) y dehidrocolato (DHC) sobre el flujo de bilis y secreción de sales, lípidos y pigmentos biliares en conejos anestesiados con pentobarbital. El GDC incrementa el flujo de bilis en mayor cuantía que el DHC, y ambos más que el TC. El diferente efecto colerético de estas sales biliares no puede explicarse por variaciones en la capacidad de formación de micelas. Sólo GDC y TC incrementan la secreción de lípidos biliares, lo que sugiere que ambas sales biliares aumentan la formación de micelas mixtas. El GDC estimula claramente, y en menor medida el TC, la excreción de pigmentos biliares, careciendo de este efecto el DHC.

References

1. BARTOS, J.: *Ann. Pharm. Fr.*, **17**, 141-144, 1959.
2. BINET, S., DELAGE, Y. and ERLINGER, S.: *Am. J. Physiol.*, **5**, E10-E14, 1979.
3. BOYER, J. L., BLOOMER, J. R.: *J. Clin. Invest.*, **54**, 773-778, 1974.
4. CAREY, M. C. and SMALL, D. M.: *Arch. Intern. Med.*, **130**, 506-537, 1972.
5. DELAGE, Y. M., DUMONT, M. and ERLINGER, S.: *Am. J. Physiol.*, **231**, 1875-1878, 1976.
6. ENGELKIN, R., GRONWALL, R. and ANWER, M. S.: *Am. J. Vet. Res.*, **41**, 355-361, 1980.
7. ERLINGER, S., DUMONT, M., BERTHELOT, P. and DHUMEAUX, D.: *Digestion*, **4**, 144, 1971.
8. ESTELLER, A., LÓPEZ, M. A. and MURILLO, A.: *Q. Jl. exp. Physiol.*, **62**, 353-359, 1977.
9. ESTELLER, A., JIMÉNEZ, R. and LÓPEZ, M. A.: *Q. Jl. exp. Physiol.*, **66**, 349-357, 1981.
10. ESTELLER, A., JIMÉNEZ, R. and LÓPEZ, M. A.: *Q. Jl. exp. Physiol.*, **66**, 359-366, 1981.
11. FOLCH, J., LEES, M. and SLOANE STANLEY, G. H.: *J. Biol. Chem.*, **226**, 497-509, 1957.
12. GORESKY, C. A., HADDAD, H. H., KLUGER, W. S., NADEU, B. and BACH, G. G.: *Can. J. Physiol. Pharmacol.*, **52**, 389-403, 1974.
13. GREGG, J. A. and POLEY, J. R.: *Am. J. Physiol.*, **211**, 1147-1151, 1966.
14. HARDISON, W. J.: *J. Lab. Clin. Med.*, **77**, 811-820, 1971.
15. HARDISON, W. J. and APTER, J. T.: *Am. J. Physiol.*, **222**, 61-67, 1972.
16. HENRY, R. J.: «Clinical Chemistry». Harper and Row Publ., New York, 1964, p. 838.
17. INVING, J. L., JOHNSTON, C. G. and KOPALA, J.: *J. Biol. Chem.*, **153**, 439-457, 1944.
18. KLAASEN, C. D.: *J. Physiol.*, **224**, 259-269, 1972.
19. LARSON, E. A., EVANS, G. T. and WATSON, C. G.: *Lab. Clin. Med.*, **32**, 481-488, 1947.
20. LEVIN, S. J., JOHNSTON, C. G. and BOYLE, G. E.: *Anal. Chem.*, **33**, 1407-1411, 1961.
21. LUPIANI, M. J., ZAMORA, S., ESTELLER, A. and LÓPEZ, M. A.: *Comp. Biochem. Physiol.*, **64A**, 185-189, 1979.
22. MALLOY, H. T. and EVELYN, K. A.: *J. Biol. Chem.*, **119**, 481-488, 1937.
23. O'MAILLE, E. R. L.: *J. Physiol.*, **302**, 107-120, 1980.

24. RUTISHAUSER, S. C. B. and STONE, S. L.: *J. Physiol.*, 245, 567-582, 1975.

25. SCHARSCHMIDT, B. F. and SCHMID, R.: *J. Clin. Invest.*, 62, 112-1131, 1978.

26. SHAW, H. M. and HEATH, T. J.: *Comp. Biochem. Physiol.*, 50A, 615-619, 1975.

27. SOLOWAY, R. D., HOFMANN, A. F., THOMAS, P. J., SCHOENFIELD, L. J. and KLEIN, P. D.: *J. Clin. Invest.*, 52, 715-724, 1973.

28. VONK, R. J., DANHOF, M., COENRAADS, T., VAN DOORN, A. B. D., KEULEMANS, K., SCAF, A. H. J. and MEIJER, D. K. F.: *Am. J. Physiol.*, 6, E524-E534, 1979.