Hepatic bile acid uptake: effect of conjugation, hydroxyl and keto groups, and albumin binding

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Abstract Hepatic extraction of trihydroxy (free, glyco- and tauro-conjugated), dihydroxy, and monohydroxy bile acids has been evaluated in single pass liver perfusion experiments in rats. The percentage of each bile acid bound to albumin was also evaluated by equilibrium dialysis. Conjugation increased bile acid liver extraction, without relevant differences in the percentage of bile acid bound to albumin. Among the free bile acids, trihydroxy bile acids were more efficiently cleared by the liver than the dihydroxy acids, and the latter more than monohydroxy bile acids. 7-Ketolithocholic acid uptake was slightly less than that of cholic acid. Conversely, among dihydroxy bile acids, the percentage of the bile acid bound to albumin decreased from lithocholic acid to cholic acid. Decreasing the albumin concentration in the medium, and hence the fraction of the bile acid bound to albumin, resulted in an increase in bile acid liver extraction. Therefore, besides differences in the chemical structure of bile acids, the extent of bile acid-albumin binding may be a determinant in bile acid liver uptake.—**Aldini, R., A. Roda, A. M. M. Labate, G. Cappelleri, E. Roda, and L. Barbara.** Hepatic bile acid uptake: effect of conjugation, hydroxyl and keto groups, and albumin binding. *J. Lipid Res.* 1982. **23:** 1167–1173.

Supplementary key words equilibrium dialysis

Bile acids (BA) are present in serum at low levels in healthy fasting subjects, due to their efficient hepatic uptake and small intestinal input. The information so far available on the hepatic BA uptake indicates that the BA share a carrier-mediated transport system (1–4) operating far from saturation at physiological levels of BA in the portal blood. Quite recently (5), receptors to BA have been identified and characterized on the liver cell membranes.

Trihydroxy BA present a maximal transport capacity approximately twice that of dihydroxy BA, independent of conjugation (1). Furthermore, different rates of hepatic extraction have been documented among different BA in rat liver perfusion experiments, using pairs of labeled BA (6). In these experiments, uptake of taurine-conjugated cholic acid was higher than that of glycine-conjugated and unconjugated cholic acid while minor differences were found among the various free BA.

At present, it is not known whether differences in the binding affinity for the uptake sites on the liver cell membranes may entirely account for differential rates of uptake for the different BA. Since BA are present in serum bound to albumin, the tightness of the BA albumin binding was thought to influence the hepatic BA uptake, and the efficiency of the hepatic uptake has been inversely correlated to the affinity of the BA to albumin (6). Quite recently, however, evidence has been presented indicating that the uptake of fatty acids takes place by saturable interaction of a fatty acid albumin complex with a receptor for albumin (7) on the liver cell surface. This receptor may account for the efficient hepatic clearance of such substances tightly bound to albumin as BA.

The present investigation was carried out in order to get more insight into the hepatic uptake of different BA and, possibly, to better define the role of BA albumin binding as a determinant of BA liver uptake.

MATERIALS AND METHODS

Liver perfusion

Isolated liver perfusions were performed as described by Mortimore (8). Male Sprague-Dawley rats (200–250 g) were used as liver donors. The perfusate consisted of Krebs-Ringer bicarbonate, pH 7.4, containing glucose (100 mg/ml) and bovine serum albumin (3% or 0.75%, w/v) (Fraction V, essentially fatty acid free, Sigma, St. Louis, MO, A 6003). The perfusion was established without using any anticoagulant and the liver was washed with 40 ml of Krebs-Ringer bicarbonate

Abbreviations: BA, bile acid; CA, cholic acid; GCA, glycocholic acid; TCA, taurocholic acid; CDCA, chenodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; UDCA, ursodeoxycholic acid; LCA, lithocholic acid; GLCA, glycolithocholic acid; 7KLCA, 7-ketolithocholic acid.

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solution. Complete and even blanching of all liver lobes during this wash-out period indicated satisfactory perfusion. Following the wash, the liver was perfused for 30 min with a recycling system containing either 3% or 0.75% albumin before starting the perfusion studies. Oxygenation with 95% O_2 and 5% CO_2 was carried out as described by Hamilton et al. (9), using silastic tubing (Dow-Corning, Midland, MI). Perfusate temperature and pH were monitored continuously and the perfusion was discharged if the values fell outside the physiological range.

Viability of the livers was also assessed by light microscopy, bile output, and oxygen consumption. The outflow was collected at 10-sec intervals, immediately after injection of the labeled BA, and the volume was retained for analysis. Before injection, 1 ml of each solution was retained for equilibrium dialysis, in order to assess the percentage of BA bound to albumin.

Materials

The following commercially available BA were used: [24-^{14}C]taurocholic acid (52.0 mCi/mmol) (Radiochemical Centre, Amersham, England); [^{3}H(G)]glycocholic acid (1.7 Ci/mmol), [24-^{14}C]cholic acid (40 mCi/mmol), [^{3}H(G)]glycochenodeoxycholic acid (1 Ci/mmol), [11,12-^{3}H]chenodeoxycholic acid (1 Ci/mmol), [^{3}H(G)]glycolithocholic acid (1 Ci/mmol), [24-^{14}C]lithocholic acid (59 mCi/mmol), (The Radiochemical Centre, Amersham, England); [11-12-^{3}H]ursodeoxycholic acid (37 Ci/mmol) (New England Nuclear, Boston, MA). [24-^{14}C]7-Ketolithocholic acid (20 mCi/mmol) was synthesized from [^{14}C]chenodeoxycholic acid according to the procedure described by Fieser (10). Glycocholic acid and glycochenodeoxycholic acid (analytical grade) were purchased from Calbiochem (La Jolla, CA).

Purity was checked by thin-layer chromatography on silica Gel-G plates (Merck, Darmstadt, Germany) in the following solvent systems: acetic acid–carbon tetrachloride–diisopropyl ether–isoamyl acetate–n-propanol–benzene, 5:20:30:40:10:10 (v/v) for the free bile acids, and propionic acid–isoamyl acetate–water–n-propanol 75:100:25:50 (v/v) for the conjugated bile acids (11). All were shown to be greater than 98% pure.

Prior to infusion into the livers, the labeled bile acids were incubated at 37°C overnight in Krebs-Ringer bicarbonate buffer containing albumin 3% or 0.75%. Immediately before the experiments, Evans blue (1 mg/g liver) (12) was added as marker of the flow rate and recovery.

Experimental design

Two sets of experiments were carried out at a physiological (3%, w/v) concentration of albumin in the medium, in order to define the effect of the following parameters on BA liver uptake. A) Tauro-, glyco-, and free cholic acid were compared in the same liver perfusion experiments (four experiments) to study the effect of conjugation. B) Free cholic, chenodeoxycholic, lithocholic, ursodeoxycholic, and 7-ketolithocholic acid were compared in the same livers (five experiments) to examine the effect of number and arrangement of hydroxyl groups and presence of keto groups on the steroid ring.

In order to evaluate the effect of albumin concentration on hepatic BA extraction, glycine-conjugated cholic, chenodeoxycholic, and lithocholic acids were injected in the same livers and hepatic extraction was compared at 3% and 0.75% albumin concentration in the perfusate. The experiments were carried out at a tracer (four experiments) and 100-μM dose of BA (four experiments). In the latter experiments, glycolithocholic acid was not used, because of its insolubility. Since the total volume of injection was 2.5 ml, 100 μM BA represented a dose of 0.025 μmol/bolus, i.e., a mass of BA less than the saturation dose reported for taurocholate (1).

In all the experiments, the bile acids were randomly injected. Since ^{3}H-labeled bile acids were also used, in order to avoid problems of overlap, a 15-min interval was interposed between each infusion. In some experiments, in addition, the first BA infused was also infused at the end of the study, in order to check the reproducibility of uptake information. If the difference between the two experiments exceeded 2.0% of the injected dose, the experiment was discharged. A total of 0.2 μCi of each bile acid was injected in each experiment, i.e., a mass inferior to the K_m reported for taurocholate (1).

Analytical methods

Radioactivity was measured on 500 μl of each outflow sample by liquid scintillation counting in a Isocap 300 (Nuclear Chicago, now Tracor Analytic, Elk Village, IL), after addition of 15 ml of Unisolve® as scintillation solution. From the known weight and activity of the injected material, it was possible to calculate the cumulative recovery as a percentage of the dose for each labeled BA, after correcting for the Evans blue recovery. The Evans blue was directly evaluated in each sample in a Perkin Elmer Spectrophotometer at 610 nm (12). Bovine serum albumin concentration in the perfusate was measured by the method of Lowry et al. (13).

Equilibrium dialysis

Equilibrium dialysis was performed in dialysis bags washed with distilled water and then with phosphate buffer 0.1 M, pH 7.2. The dialysis systems were set up with 0.3 ml each of BA solution inside the sac and 4 ml

of buffer outside, containing an identical solution of BA free of protein. The systems were equilibrated by mechanical shaking for 24 hr at 37°C. The equilibrium was verified by the absence of a change in distribution between 20 and 24 hr (14). Two hundred μl of both inner and outer solutions were then counted for radioactivity. The radioactivity in the outer (free) and inner (bound + free) fractions was used to calculate the bound BA.

Expression of the results

The percentage of outflow was corrected for the Evans blue recovery and the liver uptake was calculated as: 100 − percentage outflow. Only intra-experiment comparisons between the extraction of different BA were considered. The statistical analysis was performed using the Student's *t* test for paired data.

RESULTS

Standardizing liver perfusion

Liver perfusion data are in **Table 1.** Similar flow rates of perfusate through the liver were established. The following observations indicated the metabolic integrity of the liver. The pH of the perfusate leaving the liver was between 7.36 and 7.44; bile output averaged 0.056 ml/g liver per hr; no significant differences in SGOT levels were observed throughout the study (12 ± 2 U/l); there was normal histology as judged by light microscopy; and oxygen consumption was constant. Only preparations that fulfilled these criteria were used for the analysis of the results.

To allow quantitative interpretation of the results, it was necessary to appraise the extent of bile acid binding to the mechanical components of the perfusion system with respect to temperature (37°C) and albumin concentration (0.75–3%, w/v). The bile acids bound to the glass systems ranged from 0.5% (3% albumin) to 1.5% (0.75% albumin). The recovery of Evans blue was 97.6 ± 2.5% (mean ± SD; range, 94–102%).

Effect of conjugation and presence of hydroxyl and keto groups

Fig. 1 shows a typical experiment, comparing taurocholic, glycocholic, and free cholic acid liver outflow

TABLE 1. Liver perfusion data

Perfusate Albumin	Perfusate Flow	Portal Vein Pressure	Bile Flow	Liver Weight
g/100 ml	*ml/min per g*	*cm H_2O*	*ml/g liver per hr*	*g*
0.75	1.2 ± 0.3	12	0.056 ± 0.004	9.95 ± 0.12
3.0	1.2 ± 0.2	12	0.054 ± 0.003	10.02 ± 0.07

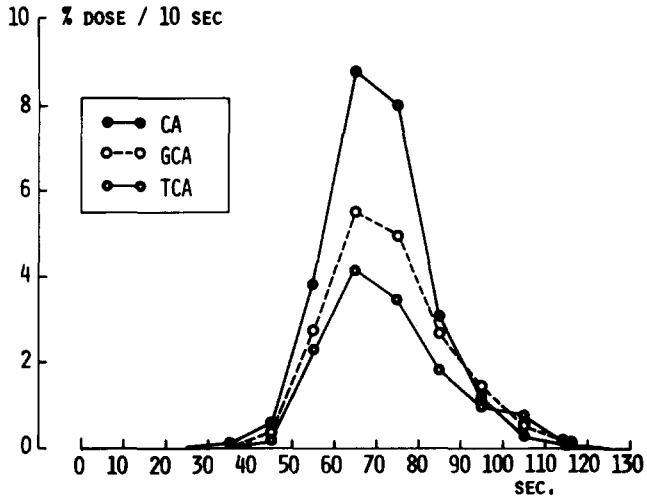

Fig. 1. Outflow profile for an experiment carried out comparing taurocholic, glycocholic, and free cholic acid in single pass uptake by liver. The experiment illustrated is number 1 in Table 1.

profiles. In all the experiments, taurocholic acid was taken up by the liver more efficiently ($P < 0.001$) than glycocholic acid and the latter more efficiently ($P < 0.001$) than cholic acid (**Table 2**).

In **Fig. 2,** the outflow profiles of unconjugated BA are shown. Cholic acid single pass extraction was significantly higher than that of 7-ketolithocholic acid ($P < 0.01$), ursodeoxycholic acid ($P < 0.005$), chenodeoxycholic acid ($P < 0.005$), and lithocholic acid ($P < 0.005$) (**Table 3**). Among the disubstituted bile acids, chenodeoxycholic and ursodeoxycholic acids were taken up to a similar extent, while 7-ketolithocholic acid extraction was slightly greater than that of the two dihydroxy bile acids ($P < 0.05$ for both BA).

Effect of albumin concentration (Table 4)

When the liver uptake of glyco-conjugated BA was compared at 3.0 g/dl albumin concentration in the perfusion medium, the uptake of glycocholic acid was higher than that of glycochenodeoxycholic acid (*P*

TABLE 2. Effect of conjugation: hepatic uptake of taurine- and glycine-conjugated and unconjugated cholic acid

	Single Pass Extraction (% dose)		
Exp.	TCA	GCA	CA
1	80	68	57
2	73	61	51
3	70	58	50
4	75	66	56
Mean	74.5	63.3	53.5
SD	4.2	4.6	3.5
P vs. TCA		0.001	0.001
P vs. GCA			0.001

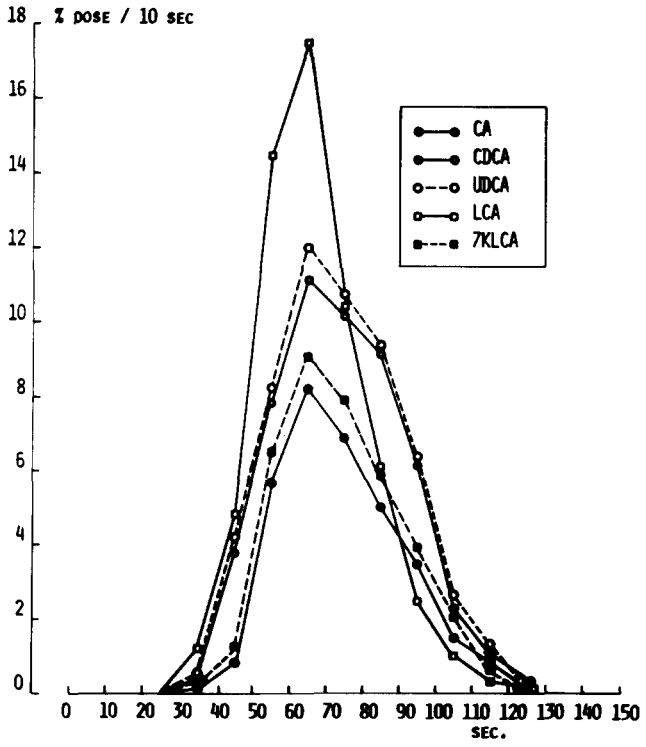

Fig. 2. Outflow profile for an experiment carried out comparing cholic, chenodeoxycholic, ursodeoxycholic, lithocholic, and 7-ketolithocholic acids in single pass uptake by liver. The experiment illustrated is number 4 in Table 3.

< 0.01) and glycolithocholic acid ($P < 0.05$). Glycochenodeoxycholic acid extraction was higher than that of glycolithocholic acid ($P < 0.05$), showing that the presence of hydroxyl groups increased the uptake both for free (see above) and conjugated BA.

A similar uptake pattern for tri-, di-, and monohydroxy glycoconjugated BA was observed when the perfusion experiments were carried out at 0.75 g/dl albumin concentration in the perfusion medium. Furthermore, the uptake of glycocholic acid was similarly higher than that of glycochenodeoxycholic acid when the two bile acids were injected as a 100-μM bolus, both at 3 g/dl albumin ($P < 0.001$) and 0.75 g/dl albumin ($P < 0.001$) concentration in the perfusion medium.

As far as the effect of albumin concentration on the uptake of the same BA is concerned, a significant ($P < 0.001$) increase in the glycocholic acid liver uptake was observed, both at tracer and 100 μM BA concentration, when the albumin concentration was lowered down from 3 to 0.75 g/dl. On the other hand, no modifications were observed between the uptake of the other two bile acids either at 3 or 0.75 g/dl concentration, when the bile acids were infused at tracer dose, while liver extraction of glycochenodeoxycholic acid at 100 μM BA concentration was significantly ($P < 0.001$) higher at 0.75 than at 3 g/dl concentration of albumin in the medium.

BA-albumin binding (Table 5)

In the presence of physiological (3%, w/v) concentrations of albumin in the medium, 48% unconjugated cholic acid was bound, while 92% chenodeoxycholic, 91% ursodeoxycholic, 85% 7-ketolithocholic, and 98% lithocholic acid were bound. Tauro- and glyco-conjugated cholic acids were 42% and 45% bound, respectively. Decreasing the albumin concentration to 0.75%, in the presence of tracer doses of BA, glycocholic acid binding was reduced to 32%, while glycochenodeoxycholic and glycolithocholic acid bindings were unchanged. In the presence of 100 μM BA concentrations in the media, however, the percentage of both glycoconjugated cholic and chenodeoxycholic acids bound was reduced by an average factor of 40% and 25%, respectively (Table 5).

DISCUSSION

The present experimental model provides further evidence that uptake of bile acids by liver is efficient for all the BA, though differences exist among the various BA. To date, different rates of hepatic extraction have been shown for conjugated cholic and free cholic acid, chenodeoxycholic acid, and deoxycholic acid, but no data are available on ursodeoxycholic and 7-ketolithocholic acids. Interest in these latter acids is now emerging in view of their use as gallstone-dissolving agents (15–17). We have documented clearcut differences in the uptake of BA; taurocholic was extracted most efficiently followed in order by glycocholic and cholic acids. Among the unconjugated BA, the hepatic uptake

TABLE 3. Effect of hydroxyl and keto groups: hepatic extraction of free cholic, chenodeoxycholic, lithocholic, ursodeoxycholic, and 7-ketolithocholic acids

	Single Pass Hepatic Extraction (% dose)				
Exp.	CA	CDCA	UDCA	LCA	7KLCA
1	52	38	48	35	47
2	48	40	41	33	44
3	46	37	36	32	39
4	46	39	41	33	44
5	79	74	73	55	76
Mean	54.2	45.6	47.8	37.6	50.0
SD	14.1	15.9	14.7	9.8	14.8
P vs. CA		0.005	0.005	0.005	0.01
P vs. CDCA			NS	0.05	0.05
P vs. UDCA				0.05	0.05
P vs. LCA					0.001

TABLE 4. Effect of albumin concentration in the medium: hepatic extraction of glycine-conjugated cholic, chenodeoxycholic, and lithocholic acids

	Bile Acids at a Tracer Concentration						Bile Acids at 100 μM Concentration			
	GCA		GCDCA		GLCA		GCA		GCDCA	
Exp.	3[a]	0.75[a]	3	0.75	3	0.75	3	0.75	3	0.75
1	44	55	39	40	32	33	64	86	57	71
2	46	59	39	43	33	33	60	81	53	68
3	79	91	74	71	55	55	57	76	51	64
4	74	86	71	76	61	63	54	76	48	62
Mean	60.7	72.7	55.7	57.5	45.2	46.0	58.8	79.8	52.3	66.3
SD	18.3	18.3	19.4	18.6	14.9	15.3	4.3	4.8	3.8	4.0
P	<0.001		NS		NS		<0.001		<0.001	
P vs. GCA			0.01	0.01	0.05	0.05			0.001	0.001
P vs. GCDCA					0.05	0.05				

[a] Albumin concentration at 3 and 0.75 g/dl.

of cholic was the highest, followed in order by 7-keto-lithocholic, ursodeoxycholic, chenodeoxycholic, and lithocholic acids. Therefore both conjugation and the number of hydroxyl groups influence hepatic BA single pass clearance.

The differences found in hepatic extraction of the free BA can be explained in terms of differences in the binding activity of albumin towards these compounds. In fact, the affinity for albumin has been shown (18) to be reduced by the introduction of polar groups into the steroid ring. Thus, the extent of binding decreases from lithocholic to cholic acid, through chenodeoxycholic (18) and ursodeoxycholic acids, and is inversely related to the hepatic extraction (**Fig. 3**).

A similar trend was observed when the hepatic extraction of glyco-conjugated BA was related to the percentage of BA bound to 3 g/dl albumin: the curve of the glyco-conjugated BA paralleled that of the free BA, but was shifted to higher values of hepatic extraction. Furthermore, decreasing the albumin concentration in the perfusate increased the liver extraction of cholic acid (both at tracer and 100 μM BA concentrations) and chenodeoxycholic acid (100 μM), probably on account of the decrease in the fraction of BA bound to albumin.

On the other hand, differences in the tightness of the BA albumin binding (14, 19) cannot entirely explain the differences in hepatic extraction of taurine- and glycine-conjugated and unconjugated BA. In fact, the conjugation with glycine and taurine greatly increases the hepatic extraction for all BA without a corresponding decrease in the percentage of BA bound to albumin. This suggests that different mechanisms, other than the binding to albumin, are involved in the overall uptake of bile acids by liver.

TABLE 5. Bile acid-albumin binding: percentage of bile acids[a] bound to bovine serum albumin

	3 g/dl Albumin		0.75 g/dl Albumin	
	n	Mean ± SD	n	Mean ± SD
CA	8	48 ± 2		
CDCA	5	92 ± 4		
UDCA	5	91 ± 5		
LCA	5	98 ± 2		
7KLCA	5	85 ± 5		
GCA	7	45 ± 2	4	32 ± 1
TCA	3	42 ± 3		
GCDCA	4	90 ± 6	4	90 ± 4
GLCA	4	97 ± 3	4	97 ± 2
GCA[b]	3	70 + 4	3	46 ± 3
GCDCA[b]	3	90 + 5	3	68 ± 4

[a] Tracer concentration for all bile acids except those indicated by footnote *b*.

[b] Bile acid concentration 100 μM.

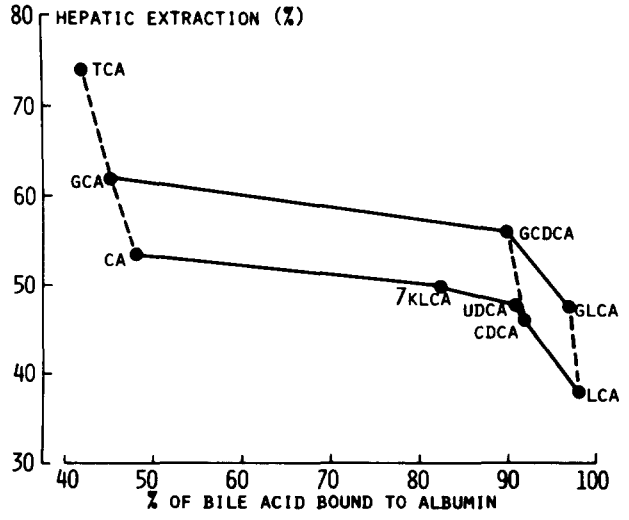

Fig. 3. Relationship between the hepatic extraction of bile acid and the percentage of bile acid bound to albumin. Solid lines represent the behavior of bile acid with different substituents; dotted lines represent the effect of conjugation for a single bile acid.

Reichen and Paumgartner (20) demonstrated that chemically different BA share a common hepatic transport system, with greater affinity for dihydroxy BA, but with higher maximal uptake velocity for trihydroxy BA. They found that conjugation increases the affinity of the BA to the receptor. Since they found that the affinity of all the bile acids studied (taurocholic, cholic, chenodeoxycholic) to albumin is of a similar order of magnitude, they concluded that the differences in the K_m observed in their studies reflect differences in the affinity of individual BA to the hepatic carrier, rather than differences in the fraction of BA bound to albumin.

Our results are in agreement with those of Reichen and Paumgartner (20), limited to the effect of conjugation on the BA–albumin binding. In fact, both the fractions of BA bound to albumin (Table 5) and the affinity of the BA to albumin (14, 18) were not greatly modified by conjugation. Therefore, differences in the binding affinities for the uptake sites on the liver cell membranes must be considered, at least as far as conjugated bile acids are concerned.

Quite recently, a surface membrane albumin receptor has been demonstrated in rat liver perfusion experiments (21); it is thought to mediate the hepatic uptake of bromosulfophthalein, bilirubin (22), and fatty acids (7). Therefore, albumin-bound fractions, not free, should be the major uptake determinant, at least for these organic anions; though other studies (23) indicate that the receptor to albumin is not involved in bilirubin hepatic uptake. On the basis of the existence of such a receptor, from kinetics analyses of the uptake of [^{14}C]oleate, in a single pass perfusion of rat liver, Weisiger, Gollan, and Ockner (7), suggested a two-component model for the uptake of fatty acids. A major component is attributable to a receptor for albumin, catalyzing the uptake of albumin-bound oleic acid, while a minor component reflects the uptake of unbound oleate. The authors considered that a similar model could be applied to other organic anions, such as bilirubin and bile acids, and could explain the efficient hepatic uptake of substances tightly bound to albumin.

Since the tightness of the albumin binding of bile acids is quite different from that of fatty acids and it differs among the BA from monohydroxy BA to trihydroxy BA, it is possible that the two components of the uptake play different roles in the overall uptake of the different BA, and that the bound fraction accounts for the uptake of almost all lithocholic acid (96–98% bound to albumin) and most of dihydroxy BA (80–90% bound), but only part of trihydroxy BA.

Moreover, Forker and Luxon (24) have demonstrated in rat liver perfusion experiments that increasing the concentration of albumin in the perfusion media reduces the uptake rate, but that this effect is much less than that predicted from concomitant changes in the BA-albumin binding. They conclude that the uptake of taurocholate depends not only upon the free bile acid but also upon the interaction between albumin and the liver cell surface.

In conclusion, both differences in the chemical structure of the bile acids and differences in the tightness of the BA binding to albumin are determinants of hepatic BA uptake. The extent to which albumin exerts its role in the uptake of bile acids by the liver and the exact mechanism involved have yet to be fully clarified.

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REFERENCES

1. Reichen, J., and G. Paumgartner. 1975. Kinetics of taurocholate uptake by the perfused rat liver. *Gastroenterology.* **68:** 132–136.
2. Glasinovic, J. C., M. Dumont, M. Duval, and S. Erlinger. 1975. Hepatocellular uptake of taurocholate in the dog. *J. Clin. Invest.* **55:** 419–426.
3. Erlinger, S., R. Poupon, J. C. Glasinovic, and M. Dumont. 1977. Hepatic uptake, storage and biliary transport maximum of bile acids in the dog. *In* Bile Acid Metabolism in Health and Disease. G. Paumgartner and A. Stiehl, editors. MTP Press, Ltd., Lancaster, England. 107–112.
4. Iser, J. H., J. S. Packer, G. F. Stening, and R. A. Smallwood. 1974. Hepatic bile salt transport in the rat—an analogue computer simulation. *Aust. N.Z. J. Med.* **4:** 305 (Abstract).
5. Accattino, L., and F. R. Simon. 1976. Identification and characterization of a bile acid receptor in isolated liver surface membranes. *J. Clin. Invest.* **57:** 496–508.
6. Hoffman, N. E., J. H. Iser, and R. A. Smallwood. 1975. Hepatic bile acid transport: effect of conjugation and position of hydroxyl groups. *Am. J. Physiol.* **229:** 298–302.
7. Weisiger, R., J. Gollan, and R. Ockner. 1981. Receptor for albumin on the liver cell surface may mediate uptake of fatty acids and other albumin-bound substances. *Science.* **211:** 1048–1051.
8. Mortimore, G. E. 1961. Effect of insulin on potassium transfer in isolated rat liver. *Am. J. Physiol.* **200:** 1315–1319.
9. Hamilton, R. L., M. N. Berry, M. C. Williams, and E. M. Severinghaus. 1974. A simple and inexpensive membrane "lung" for small organ perfusion. *J. Lipid Res.* **15:** 182–186.
10. Fieser, L. F., and S. Rajagopalan. 1949. Selective oxidation with N-bromosuccinimide. I. Cholic acid. *J. Am. Chem. Soc.* **71:** 3935–3941.
11. Hofmann, A. F. 1964. Thin-layer chromatography of bile acids and their derivatives. *In* New Biochemical Separations. J. Morr, editor. Van Nostrand, London. 283–294.
12. Stoelinga, G. B., and P. J. S. van Munster. 1967. The behaviour of Evans blue (Azo-dye-T-1824) in the body after intravenous injection. *Acta Physiol. Pharmacol. Neerl.* **14:** 391–409.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

14. Roda, A., G. Cappelleri, R. Aldini, E. Roda, and L. Barbara. 1982. Quantitative aspects of the interaction of bile acids with human serum albumin. *J. Lipid Res.* **23:** 490–495.
15. Makino, J., K. Shinozaki, and K. Yoshino. 1975. Dissolution of cholesterol gallstones by ursodeoxycholic acid. *Jpn. J. Gastroenterol.* **72:** 690–691.
16. Maton, P. N., G. M. Murphy, and R. H. Dowling. 1977. Ursodeoxycholic acid treatment of gallstones. Dose-response study and possible mechanism of action. *Lancet.* **ii:** 1297–1301.
17. Salen, A., G. S. Tint, D. Verga, and S. Shefer. 1981. The metabolism of 7-ketolithocholic acid in man. *In* Bile Acids and Lipids. G. Paumgartner, A. Stiehl, and W. Gerok, editors. MTP Press, Ltd, Lancaster, England. 97–101.
18. Rudman, D., and F. E. Kendall. 1957. Bile acid content of human serum. II. The binding of cholanic acids by human plasma proteins. *J. Clin. Invest.* **36:** 538–542.
19. O'Maille, E. R. L., T. G. Richards, and A. H. Short. 1967. The influence of conjugation of cholic acid on its uptake and secretion: hepatic extraction of taurocholate and cholate in the dog. *Am. J. Physiol.* **189:** 337–350.
20. Reichen, J., and G. Paumgartner. 1976. Uptake of bile acids by perfused rat liver. *Am. J. Physiol.* **231:** 734–742.
21. Ockner, R., R. Weisiger, and N. Lysenko. 1980. Specific and saturable binding of ^{125}I-albumin to rat hepatocytes: further evidence for a surface membrane albumin receptor. *Gastroenterology.* **79:** 1041 (Abstract).
22. Weisiger, R., J. Gollan, and R. Ockner. 1980. An albumin receptor on the liver cell may mediate hepatic uptake of sulfobromophthalein and bilirubin: bound ligand, not free, is the major uptake determinant. *Gastroenterology.* **79:** 1065 (Abstract).
23. Wolkoff, I., N. Ohni, and U. Gärtner. 1980. Uptake of bilirubin by the liver does not require an albumin receptor. *Gastroenterology.* **79:** 1068.
24. Forker, E. L., and B. A. Luxon. 1981. Albumin helps mediate removal of taurocholate by rat liver. *J. Clin. Invest.* **67:** 1517–1522.