Uptake of bile acids by perfused rat liver

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The uptake of W-labeled cholic, taurocholic, and chenodeoxycholic acid by the perfused rat liver was studied to characterize the mechanism responsible for hepatic uptake of bile acids. A rapid-injection multiple indicator-dilution technique and the three-compartment model of Goresky were employed. The kinetics of hepatic uptake of the three bile acids could be described by the Michaelis-Menten equation. The maximal uptake velocities ($V_{\text{max}}$) were 24.9 ± 2.2 (mean ± SD), 20.8 ± 1.2, and 11.4 ± 0.9 nmol/g liver for cholic, taurocholic, and chenodeoxycholic acid, respectively. The corresponding apparent half-saturation constants ($K_{\text{m}}$) were 526 ± 125, 256 ± 43, and 236 ± 48 nmol/g liver. Competitive inhibition could be demonstrated between cholate and taurocholate as well as between cholate and chenodeoxycholate. Substitution of 94% of the Na+ in the perfusion medium decreased the $V_{\text{max}}$ and the apparent $K_{\text{m}}$ of taurocholate uptake by 68 and 55%, respectively. These findings are consistent with the hypothesis that bile acids are taken up into the hepatocyte by Na+-dependent carrier-mediated transport.

Materials and Methods

Materials

Male SPF rats of the Sprague-Dawley strain (mean body wt 362 ± 46 g), purchased from the Deutsche Versuchstierfarm Hartmut-Voss, Tuttlingen, Germany, were maintained on a standard rat diet (Altromin 300R, Altromin GmbH, Lage, Germany) and tap water ad libitum. Dried, chromatographically pure bovine albumin was obtained from Behringwerke AG, Marburg/Lahn, Germany. Sodium taurocholate, cholic, and chenodeoxycholic acid were analytical A grade products from Calbiochem, Lucerne, Switzerland. They were found to be chromatographically homogeneous using several thin-layer systems (2, 10) as well as gas-liquid chromatography in the case of cholic and chenodeoxycholic acid (15). Carboxyl-$^{14}$C-labeled taurocholate sodium, cholic, and chenodeoxycholic acid were purchased from the International Chemical and Nuclear Corporation, Campus Drive, Calif. $^{99m}$Tc-labeled albumin was prepared by Dr. H. Roessler from the Division of Nuclear Medicine, Inselspital, Berne, Switzerland. Less than 2% of the pertechnetate was in its free form, as determined by equilibrium dialysis. Na$_3^{51}$CrO$_4$ was obtained from the Department of Hematology, Inselspital, Berne.

Methods

Liver perfusion experiments. The operation and perfusion techniques have been described previously (8). The preparation and cannulation of the portal vein were carried out in situ under pentobarbital anesthesia (5 mg/100 g body wt). Before cannulation of the portal vein, the rat was heparinized with Liquemarin 250 IU/100 g body wt. The interruption of the portal circulation was less than 70 s. During this period of time, the arterial blood supply of the liver was still intact. The common bile duct was cannulated with PF-10 tubing, and the bile was collected in 10-min periods in tared tubes and weighed.
HEPATIC UPTAKE OF BILE ACIDS

The perfusion medium consisted of Krebs-Ringer-bicarbonate buffer which contained 20% (vol/vol) bovine erythrocytes washed 5 times in physiologic saline, 2% (wt/vol) bovine albumin, and 0.1% (wt/vol) glucose. The perfusate was circulated with a piston pump, filtered by passage through a polyester filter (diameter 125 μm), and oxygenated with a mixture of 95% O2 and 5% CO2. The pH, standard bicarbonate, PCO2, and base excess were monitored during the perfusion experiment, and acid-base imbalance was corrected with sodium bicarbonate. Total hemoglobin was 6.34 ± 0.58 at the beginning and 6.40 ± 0.27% (wt/vol) at the end of the perfusions. The corresponding free hemoglobin concentrations were 0.03 ± 0.03 and 0.04 ± 0.03% (wt/vol).

The livers were perfused with a mean perfusion pressure of 11.5 ± 1.3 cmH2O, and a mean flow rate of 1.58 ± 0.31 ml/min·g in a temperature-constant cabinet at 37.2 ± 0.4°C. All parameters studied were related to the liver weight determined at the end of each perfusion experiment. The liver weights averaged 11.5 ± 1.3 g. The liver-to-body weight ratio was 0.032 ± 0.004.

All indicators were rapidly injected in a constant volume of 210 μl as a bolus into the portal vein. Immediately after the injections, the total hepatic venous outflow was collected in 2-s periods up to 40 s in tared tubes and weighed. Thereafter, the outflow was further collected up to 2 min in order to eliminate recirculation of the indicators.

At the beginning (after an equilibration period of 50 min) and at the end of each experiment, a mixture of 51Cr-labeled erythrocytes (1.35 μCi) and 99mTc labeled albumin (15 μCi) was given to determine the intra- and extravascular space of the liver. Between these two injections, four different doses of 14C-labeled bile acids (5 μCi) were applied in a random order together with 12.5 μCi 99mTc-labeled albumin at 20-min intervals. Applied 51Cr-labeled erythrocytes (97.6 ± 4.1%) and 95.4 ± 7.3% of the 99mTc-labeled albumin were recovered in the hepatic venous outflow. The recovery of 14C-labeled bile acids in the hepatic venous outflow and in the bile collected for 20 min was 87.9 ± 9.5%.

Kinetics of hepatocellular uptake. In five perfusion experiments, 75–25,000 nmol of cholate, dissolved in erythrocyte-free perfusion medium, were administered as described above. Similarly, chenodeoxycholate (75–25,000 nmol) was used in five experiments. Data of taurocholate uptake obtained in similar experiments had been analyzed by the two-compartment model described by Goretsky (5) for sulfobromophthalein and had previously been published (26). These data were now analyzed according to the new three-compartment model (7).

Na+ dependence of taurocholate uptake. In six experiments, a dual-perfusion system was used, which permitted to switch from the regular perfusion medium to one, where the Na+ of the Krebs Ringer bicarbonate buffer had been replaced by equimolar amounts of Li+. The erythrocytes of this perfusion medium were washed in 0.9% (wt/vol) LiCl. Due to the Na+ content of the albumin added, the final Na+ concentration in this perfusate was 7.9 ± 1.5 mM. Two doses of taurocholate (750–15,000 nmol) were studied in the same liver in the Na+ as well as in the Li+ medium, employing a crossover design. The taurocholate was given 2 min after switching from the regular to the Li+ perfusate, and the perfusion with the Li+ medium continued up to 4 min. The interval between injections was 30 min. In two perfusion experiments, tris(hydroxymethyl)aminomethane (Tris) was used instead of Li+ for Na+ replacement. The final Na+ concentration in the perfusate was 6.4 mM. The uptake of three doses of taurocholate (750; 2,500; 7,500 nmol) was studied both in the Na+ and in the Tris medium employing the same experimental design as that used in the Li+ experiments.

Inhibition studies. In six experiments, varying doses (250–7,500 nmol) of a 14C-labeled bile acid were injected together with 2,500 nmol of another, nonlabeled, inhibiting bile acid. In this fashion, the inhibition of taurocholate uptake by cholate and of cholate uptake by taurocholate and chenodeoxycholate was studied.

Equilibrium dialysis. The albumin binding of the different bile acids studied was determined by dialyzing 1 ml of different concentrations (10 μM to 10 mM) of 14C-labeled taurocholate, cholate, and chenodeoxycholate dissolved in Krebs-Ringer-bicarbonate buffer against 2% (wt/vol) bovine albumin in Krebs Ringer-bicarbonate buffer at 37°C. Since complete equilibration between the two cells was achieved by 3.5 h, dialysis was terminated at 4 h. The apparatus described by Weder and Bickel (33) and Spectrapore no. 2 membranes (Innovative AG, Olten, Switzerland; molecular weight cutoff 12–14,000) were used. The binding of bile acids to albumin in the presence of Li+ was studied employing the same system.

Analytical procedures. The pH and PCO2 in the perfusion medium were determined on an Astrup apparatus (Radiometer-Copenhagen, Denmark). 99mTc and 51Cr radioactivity were counted in a Packard 3002 Auto-Gamma spectrometer. 99mTc radioactivity was determined in the supernatant immediately after the perfusion experiment. 51Cr radioactivity was counted in whole, hemolyzed blood after decay of the 99mTc radioactivity for at least 10 half-life times. 14C radioactivity was counted in the supernatant in a Packard Tri-Carb 3380 liquid scintillation counter using Insta-Gel as scintillator. The counting efficiency was determined by the channel ratio method employing an external standard.

Mathematical and Statistical Analysis

Analysis of indicator dilution curves. The dilution curves were corrected for catheter distortion (6). The mean catheter transit time was subtracted from the mean transit time calculated by the method of Meier and Zierler (16). The distribution volumes of the different indicators were computed as the product of the mean transit time and the flow rate (16). The sinusoidal volumes were calculated according to Goretsky (4) after subtraction of the large, nonexchanging vessels.

Description of model. The uptake of bile acids was calculated using the three-compartment model proposed by Goretsky et al. (7) for the uptake of galactose. This model includes a vascular compartment (A), an extravascular compartment (B), and a cellular compartment.
In accordance with Goresky, the following assumptions have been made: 1) flow is confined to the compartment A, 2) there is no diffusion along the length in A and B, 3) flow in the sinusoids is bolus flow, 4) the diffusible indicators equilibrate instantaneously between A and B, and 5) if the total extracellular space is divided into successive theoretical elements, removal of a substance from the extracellular into the cellular space is unidirectional and takes place at a rate proportional to its plasma concentration.

In this model, the outflow profile of the extracted substance consists of two terms, namely a throughput and a returning component. The outflow fraction of the extracted substance $Q(t)_c$ is related to the outflow fraction of its appropriate reference indicator $Q(t)_r$ by the equation

$$
Q(t)_c = e^{-[(k_1 + \gamma)/C/A]} \cdot Q(t)_r + \text{returning material}
$$

In this equation, $k_1$ is the removal rate constant describing the transport of the extracted substance into the cellular compartment (dimension $t^{-1}$), $C/A$ is the ratio of the cellular to the extracellular space, $1 + \gamma$ is the ratio of the cellular to the extracellular space.

Since single injections were used, the cellular compartment may be regarded as virtually empty of bile acids. Under these conditions, no efflux of bile acids could be detected. Consequently, the term for the returning material was neglected. Equation 1 may then be transformed into

$$
\ln \frac{Q(t)_r}{Q(t)_c} = \frac{k_1 \cdot \theta}{1 + \gamma} (t)
$$

$k_1 \cdot \theta/(1 + \gamma)$ can be estimated as the slope of a plot of the natural logarithm of the ratio (albumin outflow fraction)/(bile acid outflow fraction) versus time. From the relationship of the extra- to the intravascular space, $1 + \gamma$ and, consequently, $k_1 \cdot \theta$ can be calculated.

The initial uptake velocity of bile acids was calculated as the product of $k_1 \cdot \theta$ and the dose of bile acids injected. Thus, it has the dimension of nanomoles of bile acids taken up per second and gram liver (nmol/s · g). The relationship between initial uptake velocity ($V$) and the bile acid dose ($D$) was analyzed using the Michaelis-Menten equation (17).

$$
V = \frac{V_{\text{max}} \cdot D}{K_m + D}
$$

and its linear transformation (14)

$$
D = V \cdot \frac{V_{\text{max}}}{V_{\text{max}}} - \frac{K_m}{V_{\text{max}}}
$$

The kinetic parameters, namely the apparent half-saturation constant ($K_m$) and the maximal uptake velocity ($V_{\text{max}}$), were computed nonlinearly according to Wilkinson (35) in an iterative way until stability of both parameters was attained. The uptake velocities ($V$) were weighted by the reciprocal of their variance (35). For statistical comparison of different kinetics, a linear transformation of the Michaelis-Menten equation (14) was used.

Analysis of equilibrium dialysis experiments. The binding parameters of the different bile acids to albumin were calculated using the SIGM-2-program of Pliska (25). It included calculation of the binding parameters in a first approximation by the Lineweaver-Burke equation (14)

$$
\frac{1}{V_c} = \frac{1}{P \cdot (K_{\text{ass}} \cdot c_b)^h} + \frac{1}{P}
$$

where $c_b$ represents the concentration of bile acids bound to albumin, $c_b$ the free bile acid concentration, $K_{\text{ass}}$ the association constant, $P$ the maximal binding capacity $Z_{\text{max}}$ multiplied by an arbitrary factor, and $h$ the Hill coefficient. With the parameters derived by equation 5, the program was iterated with the Hill equation 6 (9), until stability of the parameters was reached

$$
\log \frac{P - c_b}{c_b} = h \cdot \log c_b + h \cdot \log K_{\text{ass}}
$$

Statistical procedures. Regression analysis was performed by the method of least squares. Means of two samples as well as regression lines were compared by the Student $t$ test after testing the equality of variances by the $F$ test. The goodness of the fitting procedures was expressed by the $F$ value representing the ratio (regression mean square)/(residual mean square). $P < 0.05$ was regarded as significant. All results, unless otherwise stated, are expressed as the mean ± standard deviation.

RESULTS

Kinetics of Hepatocellular Uptake

The bile acid doses, the spaces of distribution of the different indicators, and other parameters for cholate and chenodeoxycholate are given in Tables 1 and 2, respectively. As described earlier for taurocholate (26), both bile acids distributed in a space not significantly different from that of albumin. The cholate space averaged 0.216 ± 0.032 ml/g, and the corresponding albumin space was 0.216 ± 0.035 ml/g. The values obtained in the chenodeoxycholate uptake experiments were 0.235 ± 0.036 and 0.237 ± 0.035 ml/g, respectively. Thus, albumin appeared to be an appropriate reference substance for bile acids, and a plot of the natural logarithm of the ratio (albumin outflow fraction)/(bile acid outflow fraction) versus time resulted in a straight line (Fig. 1). The same behavior could be demonstrated for chenodeoxycholate. The distribution space of neither cholate nor chenodeoxycholate changed with increasing dose or time (Tables 1 and 2). Table 3 contains the data for taurocholate uptake analyzed according to the new, three-compartment model. The removal rate constant of bile acids decreased nonlinarily with increasing dose. The relationship between the bile acid dose and the initial uptake velocity could adequately be described by a rectangular hyperbola obeying the Michaelis-Menten equation (17) (Fig. 2-4).
The apparent $K_m$ were 258 ± 43, 526 ± 125, and 236 ± 48 nmol/g for taurocholate, cholate, and chenodeoxycholate, respectively. The corresponding $V_{\text{max}}$ were 20.8 ± 1.2, 24.9 ± 2.2, and 11.4 ± 0.9 nmol/s·g.

Whereas the $V_{\text{max}}$ of the unconjugated cholate and the conjugated taurocholate were similar, it was considerably smaller for chenodeoxycholate. The $K_m$, however, was about 100% higher for cholate than for taurocholate.

Sodium Dependence of Taurocholate Uptake

As can be seen from Table 4, none of the spaces determined changed significantly in the presence of sodium. It amounted to 20.8 ± 1.2 and 21.2 ± 2.9 nmol/s·g.
TABLE 3. Parameters derived from taurocholate dilution curves

<table>
<thead>
<tr>
<th>Dose, nmol</th>
<th>Liver Weight, g</th>
<th>Erythrocyte Space, ml/g</th>
<th>Albumin Space, ml/g*</th>
<th>Taurocholate Space, ml/g*</th>
<th>$k_1$, 1/s</th>
<th>$k_2$, l + y*</th>
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<td>75</td>
<td>10.7</td>
<td>0.143</td>
<td>0.216</td>
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<td>0.236</td>
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<tr>
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<td>0.143</td>
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<td>0.223</td>
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<td>0.148</td>
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* Related to grams of liver.  † $k_1$, removal rate constant; $v$, ratio cellular/vascular compartment; $y$, ratio extravascular/vascular compartment. $k_1$, $y$, fractional clearance of taurocholate (dimensions of milliliters plasma transported per second per milliliter sinusoidal plasma).

FIG. 2. Relationship between dose and uptake velocity of cholate. Rectangular hyperbola ($V = 24.9 \cdot D/526 + D; F = 8.51; n = 20$) was computed according to Wilkinson (35).

in the absence and in the presence of cholate, respectively ($0.95 > P > 0.90$). Thus, cholate inhibited the uptake of taurocholate competitively, as illustrated in Fig. 6.

Competitive inhibition was also observed, when an inhibiting dose of 2,500 nmol of taurocholate was given together with varying doses of cholate. Thus, the apparent $K_m$ rose from 526 + 125 to 759 + 219 nmol/g (0.05 > $P$ > 0.025), whereas the $V_{max}$ was 24.9 ± 2.2 and 23.6 ± 7.4 in the absence and in the presence of taurocholate, respectively.

Similar results were obtained when an inhibiting dose of 2,500 nmol of chenodeoxycholate was given together with cholate: the apparent $K_m$ rose from 526 + 125 to 874 + 129 nmol/g ($P < 0.001$), whereas the $V_{max}$ was virtually unaffected (24.9 ± 2.2 in the absence and 22.9 ± 2.9 in the presence of chenodeoxycholate).

Albumin Binding of Bile Acids

The fraction of bile acid bound to albumin at different bile acid concentrations is shown in Fig. 7. The maximal fraction of chenodeoxycholate, cholate, and taurocholate bound to albumin was 92.3 ± 1.9, 76.5 ± 1.1, and 41.8 ± 2.9%, respectively; this was mainly due to differences in the maximal binding capacity and to the heterogeneity of the binding sites rather than to differences in the affinities, as can be seen from Table 6.

**DISCUSSION**

The perfused rat liver offers several advantages for the study of bile acid uptake. Thus, competition of endogenous bile acids and other substances for hepatic uptake is excluded. Sampling errors and recirculation of the indicators are eliminated by collection of the total hepatic venous outflow. Furthermore, important parameters such as portal blood flow and pressure can be controlled throughout the experiments. One of the main advantages, however, is the possibility to vary the composition of the perfusion medium to study, for instance, sodium dependence of transport processes.

Studies of the kinetic processes involved in the uptake
TABLE 4. Influence of 

<table>
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<tr>
<th>Dose, nmol</th>
<th>Liver Weight, g</th>
<th>Perfusion Medium*</th>
<th>Erythrocyte Space, ml/g</th>
<th>Albumin Space, ml/g</th>
<th>Bile Acid Space, ml/g</th>
<th>$k_1 \cdot \theta$</th>
<th>$1 + \gamma'$</th>
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<td>0.166</td>
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* The Na⁺ medium contained 140 mM Na⁺; the Li⁺ medium only 8 mM Na⁺.  
† Related to grams of liver.  
‡ $k_1 \cdot \theta$, removal rate constant; $\theta$, ratio cellular/vascular compartment; $\gamma$, ratio extravascular/vascular compartment. $k_1 \cdot \theta$, fractional clearance of taurocholate uptake (dimensions of milliliters plasma transported per second per milliliter sinusoidal plasma).

FIG. 5. Plot of uptake kinetics of taurocholate in regular perfusion medium containing 140 mM Na⁺ (○) and in a medium containing only 8 mM Na⁺ (▲), rest of Na⁺ being replaced by Li⁺. Uncompetitive inhibition is evident as a decrease in apparent $K_m$ (intercept on abscissa) as well as of $V_{max}$ (reciprocal of slope), ratio $K_m/V_{max}$ (intercept on ordinate) being virtually unchanged.

of substances at the hepatic cell surface have been greatly facilitated by multiple indicator-dilution techniques. The two-compartment model introduced by Goresky for studies of sulfobromophthalein uptake by single injections (5) has later been extended by Goresky et al. (7) to a three-compartment model for investigation of galactose uptake by constant infusions. This model offered a better description of the distribution phenomena of the indicators within the liver and has, therefore, been adapted for single injections in studies of taurocholate uptake in the dog by Glasinovic et al. (3). An additional advantage of the three-compartment model obviously would be that a component of material returning from the liver could be calculated. No use of this advantage could be made, since the observation of a single exponential relationship between the outflow fraction of albumin and of bile acids excluded the presence of a returning component. In the present studies of

TABLE 5. Competitive inhibition of bile acid uptake

<table>
<thead>
<tr>
<th>Dose, nmol</th>
<th>Liver Weight, g</th>
<th>Erythrocyte Space, ml/g</th>
<th>Albumin Space, ml/g</th>
<th>Bile Acid Space, ml/g</th>
<th>$k_1 \cdot \theta$</th>
<th>$1 + \gamma'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>12.0</td>
<td>0.093</td>
<td>0.130</td>
<td>0.136</td>
<td>0.062</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>11.8</td>
<td>0.095</td>
<td>0.132</td>
<td>0.136</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td>12.0</td>
<td>0.091</td>
<td>0.125</td>
<td>0.132</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td>11.8</td>
<td>0.096</td>
<td>0.155</td>
<td>0.157</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>2,500</td>
<td>12.0</td>
<td>0.093</td>
<td>0.114</td>
<td>0.126</td>
<td>0.088</td>
<td></td>
</tr>
<tr>
<td>2,500</td>
<td>11.8</td>
<td>0.096</td>
<td>0.155</td>
<td>0.157</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>7,500</td>
<td>12.0</td>
<td>0.091</td>
<td>0.127</td>
<td>0.128</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>7,500</td>
<td>11.8</td>
<td>0.095</td>
<td>0.150</td>
<td>0.153</td>
<td>0.029</td>
<td></td>
</tr>
</tbody>
</table>

* Related to grams of liver.  
‡ $k_1 \cdot \theta$, removal rate constant; $\theta$, ratio cellular/vascular compartment; $\gamma$, ratio extravascular/vascular compartment. $k_1 \cdot \theta$, fractional clearance of taurocholate uptake (dimensions of milliliters plasma transported per second per milliliter sinusoidal plasma).  
† Dose of inhibiting bile acid: 2,500 nmol.

FIG. 6. Plot of taurocholate uptake kinetics in absence of inhibitor (○) and in presence of 2,500 nmol cholate (▲). Competitive inhibition is evident as an increase in apparent $K_m$ (intercept on abscissa) without a change in $V_{max}$ (reciprocal of slope).
bile acid uptake, the single-injection technique has been preferred to the constant-infusion technique because of the hemolytic effect of bile acids at high concentrations. In addition, this technique of studying initial bile acid uptake has the advantage that the occurrence of preloading phenomena may be excluded.

Albumin appeared to be an appropriate reference substance for the study of hepatic bile acid uptake, since all the bile acids studied distributed in a space equivalent to that of albumin. Consequently, a plot of the natural logarithm of the ratio (albumin outflow fraction)/(bile acid outflow fraction) versus time resulted in a straight line without further mathematical manipulations. In the dog, Glasimovic et al. (3) found a greater space of distribution for taurocholate than for albumin. These authors assumed that the fraction of taurocholate not bound to albumin would distribute in a space greater than the albumin space. The difference between their findings and ours remains unclear, but may be related to differences in species and methodology.

Previous studies of hepatocellular uptake of taurocholate (3, 26) are consistent with carrier-mediated transport of bile acids through the sinusoidal membrane of the hepatocyte, but further evidence for this hypothesis is lacking. Two findings of the present investigations, namely apparent Na+ dependence and competitive inhibition of taurocholate uptake, support the concept that a membrane carrier mechanism is responsible for bile acid uptake by the liver. Na+ dependence so far has only been reported for concentrative membrane transport processes (30). The decrease of taurocholate uptake found in the absence of Na+ is unlikely to result from toxic damage by Li+, since essential parameters such as blood flow and oxygen consumption were virtually unchanged. Moreover, uptake of a subsequent dose of taurocholate in the regular perfusion medium was unaffected. The contention that the depression of taurocholate uptake is specifically related to the absence of Na+ rather than to some other effect of Li+ is further supported by the finding of a similar effect when Na+ was replaced by Tris. Although the above findings may be interpreted as suggestive evidence for Na+ dependence of hepatic taurocholate uptake, complete characterization of Na+-dependent cotransport would have to include information on the relationship between the Na+ concentration and bile acid uptake. Since the apparent $K_m$ exhibits a behavior opposite to that which characterizes other Na+-dependent systems, such a study would be of considerable interest. The effect of Na+ replacement by Li+ resembled an uncompetitive type of inhibition of taurocholate uptake. Such a type of inhibition has been observed in enzyme chemistry, when the inhibitor combined with the enzyme-substrate complex (36). Whether Li+ and Na+ combine with the bile acid-carrier complex in a similar fashion remains to be elucidated.

Since competition for uptake between conjugated and unconjugated as well as between di- and trihydroxy bile acids could be demonstrated, it may be assumed that chemically different bile acids share a common transport system for hepatic uptake. This pathway appears to be different from that by which anionic dyes such as indocyanine green enter the hepatocyte (23).

Kinetic differences in the transport of bile acids can be observed. Since the maximal uptake velocity of the trihydroxy bile acids, cholate and taurocholate, were approximately twice that of the dihydroxy bile acid, chenodeoxycholate, it may be concluded that the maximal uptake capacity is mainly influenced by the number of hydroxyl groups. The apparent half-saturation constant, $K_m$, was similar for taurocholate and chenodeoxycholate, whereas it was about 100% higher for unconjugated cholate. Thus, it appears that dihydroxy bile acids have a greater affinity to the transport system than trihydroxy bile acids, when unconjugated chenodeoxycholate and cholate are compared. The effect of conjugation has only been investigated for cholate: it was found to increase the affinity.

Under physiological conditions, the hepatic uptake system operates far from saturation. For example, the plasma concentration leading to half-saturation of the uptake mechanism was 1.1, 2.4, and 1.0 mM for taurocholate, cholate, and chenodeoxycholate, respectively, whereas the normal concentration of bile acids in the portal vein of the rat is about 0.17 mM (18). As pointed out previously (26), the $K_m$ values must be considered as minimal estimates, since their calculation is based on the assumption that the bile acid dose is evenly distrib-

**Table 6. Parameters of bile acid binding to albumin**

<table>
<thead>
<tr>
<th>Bile Acid</th>
<th>Maximal Binding Capacity $Z_{max}$, mol</th>
<th>Association Constant $K_{assoc}$, liters/mol²</th>
<th>Hill Coefficient $h^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurocholate</td>
<td>$4.0 \pm 0.1$</td>
<td>$9.5 \times 10^7$</td>
<td>$0.94 \pm 0.03$</td>
</tr>
<tr>
<td>Cholate</td>
<td>$13.3 \pm 0.2$</td>
<td>$4.1 \times 10^6$</td>
<td>$0.74 \pm 0.02$</td>
</tr>
<tr>
<td>Chenodeoxycholate</td>
<td>$19.4 \pm 0.3$</td>
<td>$6.2 \times 10^5$</td>
<td>$0.54 \pm 0.01$</td>
</tr>
</tbody>
</table>

* ±95% confidential limits.
HEPATIC UPTAKE OF BILE ACIDS

uted along the sinusoids at time 0. In reality, the dose is confined to a traveling concentration wave occupying a smaller proportion if the sinusoidal length (5).

The maximal uptake capacity, $V_{\text{max}}$, of all three bile acids studied exceeded the steady-state excretory transport maximum of taurocholate, determined in the perfused rat liver (22), and it may, therefore, be concluded that hepatic uptake is not the rate-limiting step in the overall transport of bile acids from blood into bile. The disproportion between the uptake and excretory capacities however, is smaller for bile acids (approximately 6:1) than for sulfobromophthalein (60:1) (5) and for indocyanine green (38:1) (23).

It must be kept in mind that only apparent $K_m$ and $V_{\text{max}}$ have been determined. These parameters are results of the true affinity and capacity of the transport system and of the binding proteins in the perfusate. Therefore, in all studies dealing with the kinetics of hepatic uptake, the effect of protein binding on the transport parameters should be considered. It may be assumed that the effect of albumin binding on the maximal uptake capacity is small, since only 2.6, 1.5, and 18.6% of taurocholate, cholate, and chenodeoxycholate were bound at concentrations leading to 98% saturation of the uptake system (closed arrows in Fig. 7). The $K_m$ is probably more affected by albumin binding of bile acids, since 31.7, 41.2, and 81.9% of taurocholate, cholate, and chenodeoxycholate were bound to albumin at concentrations leading to half-saturation of the uptake system (open arrows in Fig. 7). The differences in the fractions bound to albumin appear to be due to differences in the maximal binding capacity rather than to differences in the affinities. Another contributing factor may be the heterogeneity of the binding sites as expressed by the Hill coefficients (21, 25). Since the affinity of all bile acids studied is of the order of magnitude of $10^8$ liters/mol, it may be assumed that the effect of albumin binding on the maximal uptake capacity is small, since only 2.6, 1.5, and 18.6% of taurocholate, cholate, and chenodeoxycholate were bound at concentrations leading to 98% saturation of the uptake system (closed arrows in Fig. 7). The $K_m$ is probably more affected by albumin binding of bile acids, since 31.7, 41.2, and 81.9% of taurocholate, cholate, and chenodeoxycholate were bound to albumin at concentrations leading to half-saturation of the uptake system (open arrows in Fig. 7). The Hill coefficients, 31.7, 41.2, and 81.9% of taurocholate, cholate, and chenodeoxycholate were bound to albumin at concentrations leading to 98% saturation of the uptake system (closed arrows in Fig. 7). The $K_m$ is probably more affected by albumin binding of bile acids, since 31.7, 41.2, and 81.9% of taurocholate, cholate, and chenodeoxycholate were bound to albumin at concentrations leading to half-saturation of the uptake system (open arrows in Fig. 7). The differences in the fractions bound to albumin appear to be due to differences in the maximal binding capacity rather than to differences in the affinities. Another contributing factor may be the heterogeneity of the binding sites as expressed by the low Hill coefficients (21, 25). Since the affinity of all bile acids studied is of the order of magnitude of $10^8$ liters/mol, it may be assumed that the differences in the apparent $K_m$ reflect differences in the true affinity of individual bile acids to the hepatic carrier.

It is particularly noteworthy that several similarities exist between the transport system for bile acids of the hepatic canalicular and of the terminal ileum (11–13, 20, 24, 29, 34). Thus, sodium dependence (11, 24) and competitive inhibition phenomena (13) have been described in the rat ileum. Similar structure-transport relationships have been documented in rat (13) and human (12) ileum.

The present studies have shown that bile acids are removed from the portal circulation by a remarkably efficient transport system exhibiting a high affinity to both conjugated and unconjugated bile acids and a maximal transport capacity exceeding that reported for ileal transport (13, 29, 34). These two characteristics appear to be essential for maintaining the low concentrations of bile acids observed in peripheral blood (18, 28) in spite of large daily loads of bile acids reaching the liver during their enterohepatic circulation (34). Furthermore, the transport parameters of sinusoidal bile acid uptake may be important determinants for the bile acid concentration within the hepatocyte, which is thought to regulate cholesterol and bile acid synthesis (31, 34).

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