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HEPATIC UPTAKE AND BILIARY EXCRETION OF ORGANIC CATIONS—I

CHARACTERIZATION OF THREE NEW MODEL COMPOUNDS

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Abstract—Three quaternary ammonium compounds (QACs) with different lipophilicity, triethylmethyl ammonium iodide (TEMA), tripropylmethyl ammonium iodide (TPMA) and tri-*n*-butylmethyl ammonium iodide (TBuMA) were given as a bolus injection of 10 μmole and 1 μmole in an isolated perfused liver. TPMA and TBuMA exhibited saturation kinetics at a dose of 10 μmole, but not when 1 μmole of the agents was given. Biliary clearance of TEMA was equal to the bile flow (0.010 ml/min), whereas for TPMA and TBuMA much higher values of 0.8 ml/min and 2.2 ml/min were found respectively. Partition coefficients of TEMA, TPMA and TBuMA between *n*-octanol and Krebs-bicarbonate solution were 0.0013, 0.013 and 0.14 respectively. Liver-to-plasma concentration ratios were 4, 16 and 30 in the post-distribution phase, whereas bile-to-liver ratios were calculated to be 0.1, 1.3 and 14 respectively. The latter parameter varied roughly proportionally to the lipophilicity of the compounds. The liver/plasma concentration ratios corrected for intracellular binding exceeded a value of 12 indicating that accumulation in the liver of these agents cannot soley be explained by passive equilibration according to the membrane potential. Transport from liver into the bile of TPMA and TBuMA presumably also occurred against an electrochemical gradient. It was inferred that the small molecular weight compounds such as TEMA, can be transported from plasma into bile paracellularly by a passive process. Rapid uptake into the liver of such compounds may not lead to an appreciable biliary output and can even reduce the rate of biliary excretion. QACs with intermediate or high lipophilicity are transported by carrier mediated processes both at the level of hepatocyte uptake and bile canalicular transport.

The influence of choleresis on hepato-biliary transport of the three QACs was investigated by giving sodium taurocholate (Tc) by constant infusion of 60 μmole/hr, increasing bile flow from 9 to 16 μl/min. The biliary output of TEMA appeared to be basically unaffected, whereas the biliary excretion of TPMA and TBuMA was clearly elevated when the bile flow was increased. The stimulatory influence of taurocholate on the biliary output of the latter organic cations is explained by an increased net uptake of these agents into the liver and an increased net canalicular transport. This effect is proposed to be due to a reduced reabsorption from the biliary tree as a consequence of the higher bile flow and/or biliary micelle binding. Taurocholate increased liver-to-plasma ratios. Pharmacokinetic analysis indicated a reduction by taurocholate of the rate of liver-to-plasma transport of the quaternary ammonium compounds. This may be due to ion-pair formation between taurocholate and these agents, resulting in an increased intracellular binding and/or a reduction in the effective concentrations for the organic cation transport process. It is concluded that the three investigated quaternary agents are useful model compounds to study the various mechanisms for organic cation transport in the liver.

Hepatic transport and biliary excretion of organic cations like quaternary ammonium compounds (QACs) have been reviewed by several authors [1–3]. Transport studies with these agents have been performed in many *in vivo* experiments in intact animals [4] or *in vitro* in isolated perfused liver [3, 5], liver slices [6], hepatocytes [7–9] and liver membrane vesicles [10]. Although many different QACs were used in these studies it remained unclear how the chemical structure influenced the various transport steps involved in hepato-biliary transport. Hwang [11] proposed acetyl procainamide ethobromide (APAEB) as a useful model substance for transport studies. In our opinion APAEB has a chemical structure that is too complex for this kind of experiment, possessing an ionizable amide group beside the charged nitrogen; furthermore APAEB is protein bound, which makes interpretation of the results of the transport studies rather difficult. In a previous study [12] we found three simple aliphatic QACs to be potential model compounds for hepatic transport studies among a series of aliphatic and aromatic QACs, since they are not protein bound and are not metabolized. In addition these three QACs represented three groups of QACs as related to lipophilicity and extent of biliary excretion. Triethyl methyl ammonium (TEMA) represents compounds with a low lipophilicity which are excreted into the bile in small amounts (less than 1% of the dose) [13, 14]. Biliary clearance of these compounds is equal to the bile flow. Tri-*n*-butyl methyl ammonium (TBuMA) represents a group of relatively highly lipophilic compounds which are excreted into the bile

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in large amounts (more than 30% of the administered dose). Tripropyl methyl ammonium (TPMA) is a QAC with an intermediate lipophilicity. Its biliary excretion was about 10% of the administered dose *in vivo*. APAEB belongs to this last type of QACs.

In hepatic transport at least three different transport steps are usually distinguished: uptake into the liver across the sinusoidal membrane, transport across the cells and canalicular transport into the bile. Until now the mechanisms at the membrane level are still unexplained.

Several authors described the influence of bile salts and bile salt induced choleresis on the transport of QACs *in vivo* and *in vitro* [5, 8–10, 15–17] but the results from these studies are controversial. Two reports indicated no effect of taurocholate and dehydrocholate [5, 15] whereas in two other papers stimulating effects were described [16, 17]. Such discrepancies may be related to the dose regimes chosen for the bile salts and the organic cations. For instance at relatively high doses of bile salts inhibitory effects may be observed [17]. Furthermore, the magnitude of the dose as well as the way of administration of the organic cation may determine whether the hepatic uptake or biliary secretion step is rate limiting in the overall hepatobiliary transport and thereby may determine the outcome of the interactions with bile salts.

Eaton and Klaassen [8] studied PAEB uptake in hepatocytes. They found no significant effect on PAEB uptake into the cells by taurocholate at a taurocholate/PAEB ratio of 0.75 (75 μM taurocholate, 100 μM PAEB) nor at a ratio of 7.5. In previous studies from our laboratory Vonk *et al.* [9] used hepatocytes to study the uptake and release of APAEB in the presence of taurocholate. A concentration of 60 μM taurocholate caused a 50% decrease in the uptake of APAEB into the cells. In contrast concentrations up to 3.3 μM taurocholate failed to affect the release of APAEB from the hepatocyte.

Ruifrok [10] recently studied in our laboratory the uptake of some quaternary ammonium compounds into rat liver plasma membrane vesicles. He only observed measurable transport for organic cations which possessed a sufficient lipophilicity (*N*-methyldeptropine). Uptake of *N*-methyldeptropine into the vesicles was decreased by taurocholate (5 mM).

In the present study we investigated the influence of a taurocholate-induced choleresis on the hepatic transport steps for the model compounds TEMA, TPMA and TBuMA.

MATERIALS AND METHODS

Materials. ^{14}C-labelled TEMA, TPMA and TBuMA were synthetized as described previously [13]. Albumin was obtained from Organon Technika (Oss, The Netherlands) and all other chemicals were from E. Merck (Darmstadt). Sodium taurocholate was purchased from Fluka (Buchs, SG, FRG).

Radiochemical analysis. All ^{14}C-labelled compounds were measured by liquid scintillation counting after mixing bile, perfusion medium and homogenates with a premixed medium (Plasmasol, Packard, Groningen, The Netherlands). Quenching of each sample was corrected by external standardization. The quarternary ammonium compounds used in this study are not metabolized. Their binding to plasma proteins is negligible [14].

Liver perfusion studies. The perfusion technique was used as described before [18]. The perfusion medium consisted of a Krebs-bicarbonate solution supplemented with 1% bovine albumin. Phosphate concentration in the medium was doubled compared with previously used perfusion medium [19]. The perfusate flow was adjusted to 40 ml/min. A volume of 100 ml of perfusion medium was used in all experiments. Perfusions were performed over a 2 hr period. The doses were either 10 μmole or 1 μmole of the three QACs given as a bolus injection. The experiments (N = 3) with a dose of 1 μmole were used as control experiments. The influence of sodium taurocholate (Tc) was investigated by giving a constant infusion of 60 μmole/hr into the perfusion medium. Under these conditions a steady state Tc-plasma level of 0.16 μmole/ml was reached. Plasma samples were taken at different times and bile was collected during intervals of 5 or 10 min. At the end of the experiments the liver was homogenized in a saline solution (the volume was four times the liver weight) and a sample was submitted to liquid scintillation counting. Liver content at various time intervals was calculated by subtracting from the administered dose the amount in the perfusate plus the amount excreted into the bile. In some livers (two for each compound) subcellular distribution was studied by homogenizing the liver with 1.5 its volume of 0.15 M KCl [5, 20]. In the supernatant obtained after ultracentrifugation the free concentration was determined by ultrafiltration as reported before [5, 20].

Pharmacokinetic analysis of the data. The plasma disappearance-, liver content- and biliary excretion rate vs time curves were fitted with the computer program RUGFIT. This procedure consists of an interactive peeling of the curves on the basis of log linear regression lines, using a weighed least squares method. RUGFIT fits these curves to maximal five exponential terms with the special feature that no preassumption on the number of exponential terms is made. The choice of the best fitting exponential function is made on statistical grounds and tested on a 95% significance level according to Boxenbaum [21].

From the primary parameters (i.e. slopes and intercepts) the program then calculates the secondary pharmacokinetic parameters such as rate constants, distribution volumes, and plasma clearance. This analysis is performed according to the compartmental model which adequately explains the combined exponential patterns observed in the plasma disappearance and excretion rate curves. The goodness of fit for each individual curve is calculated within the program and is expressed as the standard deviation. In addition the standard deviation of the estimated slopes and intercepts of the individual curves are calculated. Fitting of the data of the present study showed standard deviations of maximal ± 20%. Standard errors of the mean in the rate constants (see Table 1) were less than ± 15%.

Table 1. Kinetic parameters (mean values) of triethylmethyl ammonium (TEMA), tripropylmethyl ammonium (TPMA) and tributylmethyl ammonium (TBuMA) from the plasma disappearance-, liver content- and excretion rate vs time curves

Compound	Dose (μmole)	$t_{\frac{1}{2}}\lambda_1$ (min)	$t_{\frac{1}{2}}\lambda_2$ (min)	Cl (ml/min)	Ae (2 hr) (%)
			Plasma*		
TEMA	1	1.1	>240	0	0.46
+Tc	1	1.2	>240	0	0.45
TPMA	1	5.3	>240	0.76	6.6
+Tc	1	4.9	>240	0.86	10.3†
TBuMA	1	4.6	138	2.2	52.1
+Tc	1	6.3	121	3.5†	69.1†
		Liver			
TEMA	1	3.5	>240		
+Tc	1	1.7	>240		
TPMA	1	7.7	>240		
+Tc	1	5.9	>240		
TBuMA	1	3.9	72.9		
+Tc	1	3.8	44.5†		
			Bile		
TEMA	1	15.1	61.2	0.047	10.0
+Tc‡	1	—	—	0.044	16.4†
TPMA	1	16.5	101	0.71	9.9
+Tc	1	22.2	48.5†	1.03†	15.3†
TBuMA	1	15.0	63.9	7.5	8.1
+Tc	1	10.1	24.6†	12.3†	15.2†

Half lives ($t_{\frac{1}{2}}\lambda_z$), clearance from the perfusion medium (Cl = D/AUC), percentage excreted into the bile (Ae), maximal excretion rate (ER_{max}) and bile flow are given for a dose of 1 μmole and when a taurocholate infusion (TC) was given.

S.E.M. are less than ±15%.

* Plasma disappearance curves are fitted according to:

$$C = C_1 \times e^{-\lambda_1 \times t} + C_2 \times e^{-\lambda_2 \times t},$$

where C represents the plasma concentration.
Liver and excretion rate curves are fitted according to:

$$A = -A_1 \times e^{-\lambda_1 \times t} + A_2 \times e^{-\lambda_2 \times t},$$

where A represents the liver content (for the liver curve) or dAe/dt in the biliary excretion rate curve.

† Statistically different from controls ($P < 0.05$).

‡ These data could not be fitted with the program RUGFIT.

RESULTS

Pharmacokinetic pattern

TEMA, TPMA and TBuMA showed a very different pharmacokinetic pattern, compared to each other. The pharmacokinetic data were summarized in Table 1. Since it was found, as will be described later, that the dose of 10 μmole showed saturation kinetics, the usual pharmacokinetic analysis in principle is not allowed and the half lives given in the text for D = 10 μmole must therefore be considered only as indicative, to give an impression of the observed differences.

Triethylmethyl ammonium

Plasma disappearance–time curve of TEMA (Figs. 1 and 4) were fitted according to a two exponential equation in which procedure the extremely long half life of the last phase was taken as two times the duration of the experiment (>240 min) (see Table 1). The initial distribution half life was 3.4 min when 10 μmole was given and 1.1 min for a dose of 1 μmole. The rate of uptake of TEMA into the liver at the latter dose was so fast that it was practically flow limited. Half lives of the ascending phase of the liver curves were almost equal for both doses. The percentages of TEMA stored in the liver at the end of the experiment were 33% and 43% of the administered dose for 10 μmole and 1 μmole respectively. The biliary output of TEMA increased with increasing dose, but the relative amount excreted via the bile was only 0.46% of the dose in both experiments. The biliary clearance of TEMA, calculated according to $(dAe/dt)/C_{plasma}$, amounted to 0.0072 ml/min (D = 10 μmole) and 0.0085 ml/min (D = 1 μmole), being in the range of the bile flow. (dAe/dt represents the amount excreted during dt and C_{plasma} is the plasma concentration at the midpoint of that time interval).

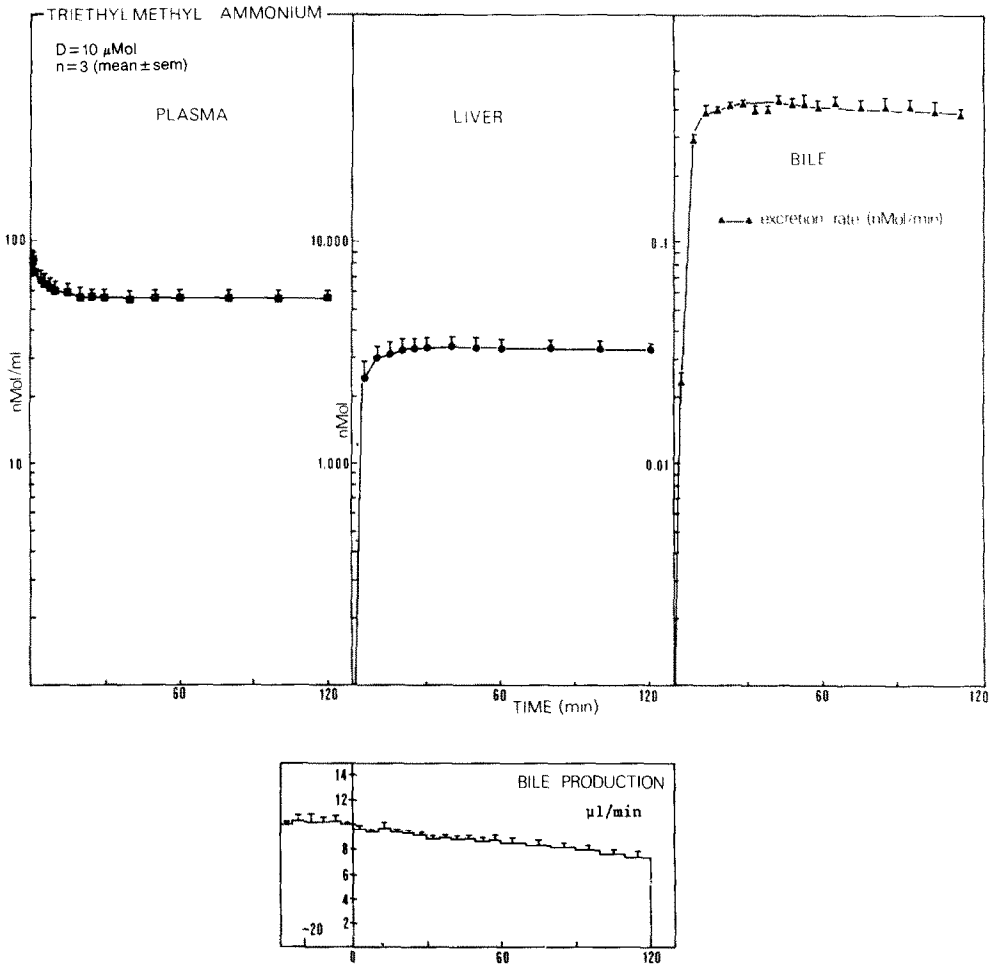

Fig. 1. TEMA kinetics in the isolated perfused rat liver after a bolus injection of 10 μmole. Concentrations in plasma (left), amounts in the liver (middle), biliary excretion rate (right) and bile flow (middle) are indicated. The amounts excreted into the bile are expressed as nmole substrate excreted per minute and are plotted at the midpoint of the time interval of sampling (5 or 10 min). The curves depicted are the mean ± S.E.M. of three separate experiments.

Tripropylmethyl ammonium

Plasma disappearance vs time curves of TPMA demonstrated more pronounced differences at the two doses than was found for TEMA (Figs. 2 and 5). This was reflected in the distribution half lives (Table 1), an apparent $t_{\frac{1}{2}}$ of 23.6 min for D = 10 μmole and a $t_{\frac{1}{2}}$ of 5.3 min for D = 1 μmole. The liver content–time curves showed a pronounced ascending phase, indicating a slow equilibration of the liver and plasma compartments. This phase had an apparent half life of 19.1 min with the dose of 10 μmole and 7.7 min for the dose of D = 1 μmole. The percentages of the QAC stored in the liver at the end of the experiments were 41% and 53% of the administered dose respectively. The biliary excretion rate did not increase proportionally with the dose: the amounts excreted via the bile were 3.45% of the dose with D = 10 μmole and 6.60% of the dose when 1 μmole was given.

Tributylmethyl ammonium

The pharmacokinetic profile of the third cation TBuMA also showed saturation kinetics in the dose range employed (Figs. 3 and 6, and Table 1). Initial half lives were 13.7 min and 4.6 min for D = 10 μmole and D = 1 μmole respectively. The liver content–time curves also showed different patterns for the two doses. Both curves had an ascending phase but with different half lives: 15.6 min for D = 10 μmole and 3.9 min for D = 1 μmole. The amounts of the QAC stored in the liver at the end of the experiments were 35% of the dose (D = 10 μmole) and 25% of the dose (D = 1 μmole), while 45% and 62% of the dose was present in the liver at T_{max} for 10 and 1 μmole respectively. The biliary excretion rate vs time curves showed the same profile as the respective liver curves: the same T_{max} values and ascending phases. The amounts excreted into the bile during 2 hr perfusion differed: 28% of the dose

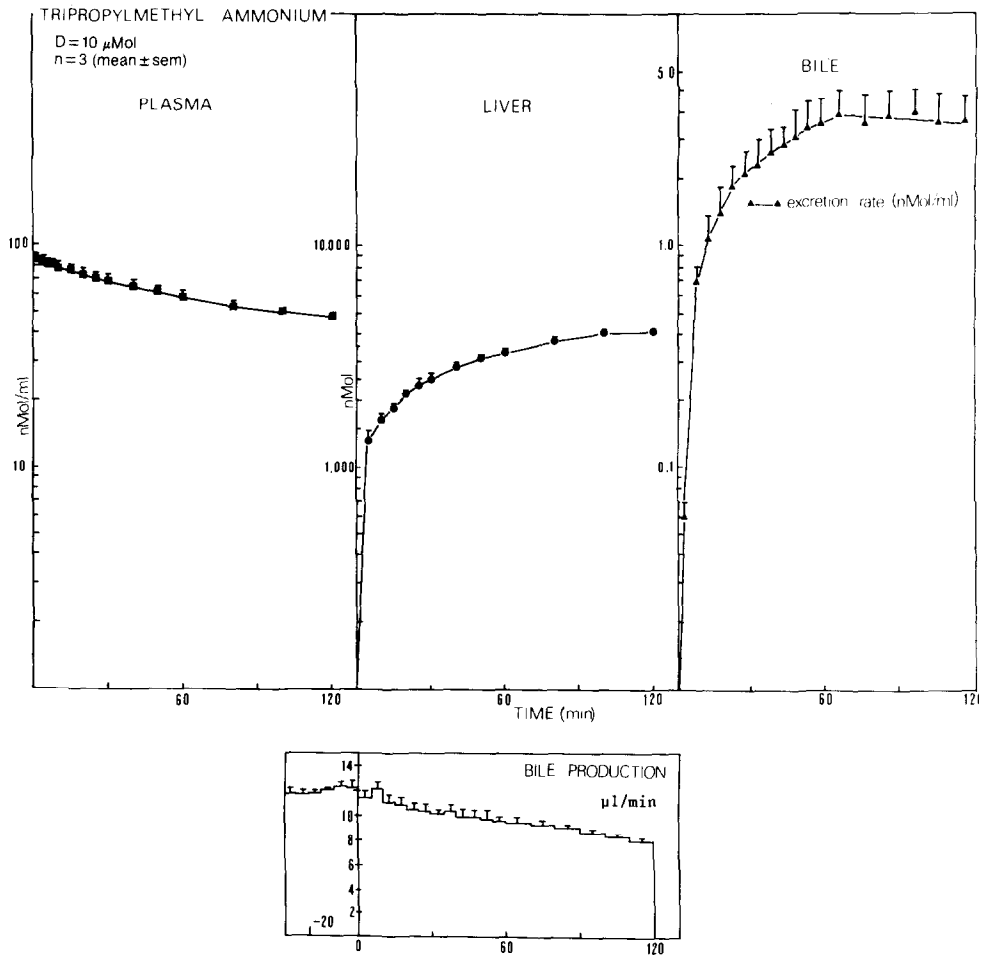

Fig. 2. TPMA kinetics in the isolated perfused rat liver after a bolus injection of 10 μmole. Details are as indicated in the legend of Fig. 1.

for D = 10 μmole and 52% of the dose for D = 1 μmole.

The bile flow remained fairly constant throughout the experiments for all three compounds at the two doses (about 9 μl/min).

Taurocholate infusion

Plasma disappearance. A constant infusion of sodium taurocholate had a similar effect on the plasma disappearance of the three QACs: all plasma levels were lower than the control values, although for TPMA the difference was very small (Figs. 7–9). The half lives were presented in Table 1. Only TBuMA showed a smaller terminal half life.

Liver uptake. A decrease of the plasma level was accompanied by a clear increase in liver content of TEMA and no effect on liver content in the case of TPMA was shown. With TBuMA even a decrease of the content of the liver was seen. The maximal amount of TBuMA in the liver was equal to the control, but the amount in the liver after two hours was much lower (12.5%). This was also reflected in the slope of the terminal phase of the hepatic content and biliary excretion rate curves.

Biliary excretion. The biliary excretion rate curve of TEMA during Tc infusion matched the control curve, in spite of an increased bile flow (mean 16.4 μl/min ± 3.8 S.E.M., compared with 10.0 ± 1.4 for the control). In contrast the excretion rate (ER) of TPMA increased proportional to the bile flow (bile flow, 15.3 ± 1.2 μl/min, compared to 9.9 ± 1.3 μl/min for the control; excretion rate, 1.03 nmol/min and 0.71 nmol/min respectively), but the shape of the excretion curve was not changed. The amount excreted into the bile was almost doubled (10.3% of the dose, compared with 6.6% of the dose for the control). A similar enhancing effect was shown for TBuMA. The time of maximal excretion (15–20 min) was shorter than the control and the maximal excretion rate also increased (12.3 nmole/min) compared with the excretion rate from the control experiment (7.5 nmole/min). The mean bile flow in the TBuMA experiments was 15.2 ± 0.5 μl/min (8.1 ± 0.7 μl/min for the control). The plasma clearance of TBuMA also increased as did the amount excreted within two hours perfusion (69.1% of the dose, compared with 52.1% for the control).

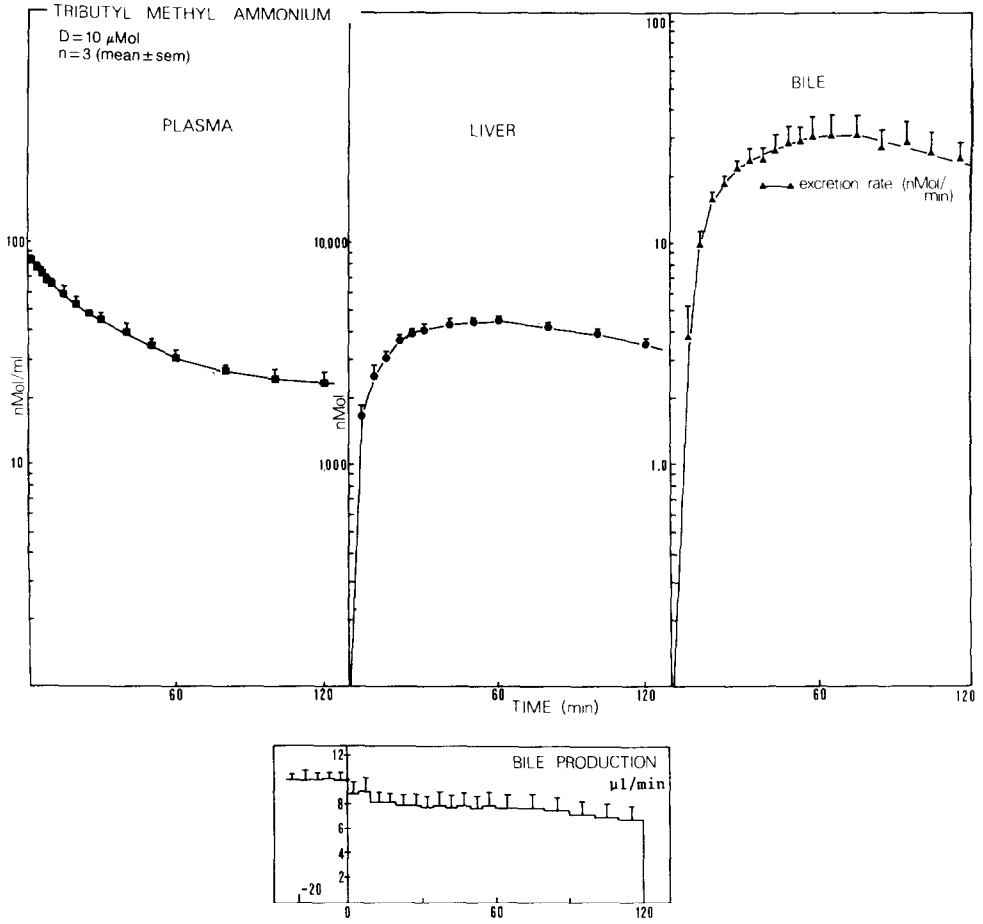

Fig. 3. TBuMA kinetics in the isolated perfused rat liver after a bolus injection of 10 μmole. Details are as indicated in the legend of Fig. 1.

Table 2. Ratios of concentrations in the perfusion medium (P), the liver (L) and the bile (B) in post-distribution phase of the perfusion (i.e. from $t = 30$ to $t = 120$ min)

Compound	Dose (μmole)	L/P	B/L	B/P
TEMA	10	6.3 ± 0.1	0.14 ± 0.01	0.83 ± 0.05
	1	9.0 ± 1.0*	0.10 ± 0.01*	0.90 ± 0.04
+Tc	1	19 ± 1†	0.04 ± 0.01†	0.73 ± 0.07
TPMA	10	6.2 ± 1.5	1.1 ± 0.1	6.7 ± 2.2
	1	16 ± 1*	1.3 ± 0.1	21 ± 5*
+Tc	1	17 ± 1	1.4 ± 0.2	23 ± 4
TBuMA	10	16 ± 2	7.7 ± 0.8	104 ± 56
	1	30 ± 4	14 ± 2*	404 ± 96*
+Tc	1	29 ± 6	12 ± 3	377 ± 137

The values of this phase were averaged.
Mean values ± S.E.M. are given.
* Statistically different compared with 10 μmole dose ($P < 0.05$).
† Statistically different compared with controls (1 μmole, $P < 0.05$).

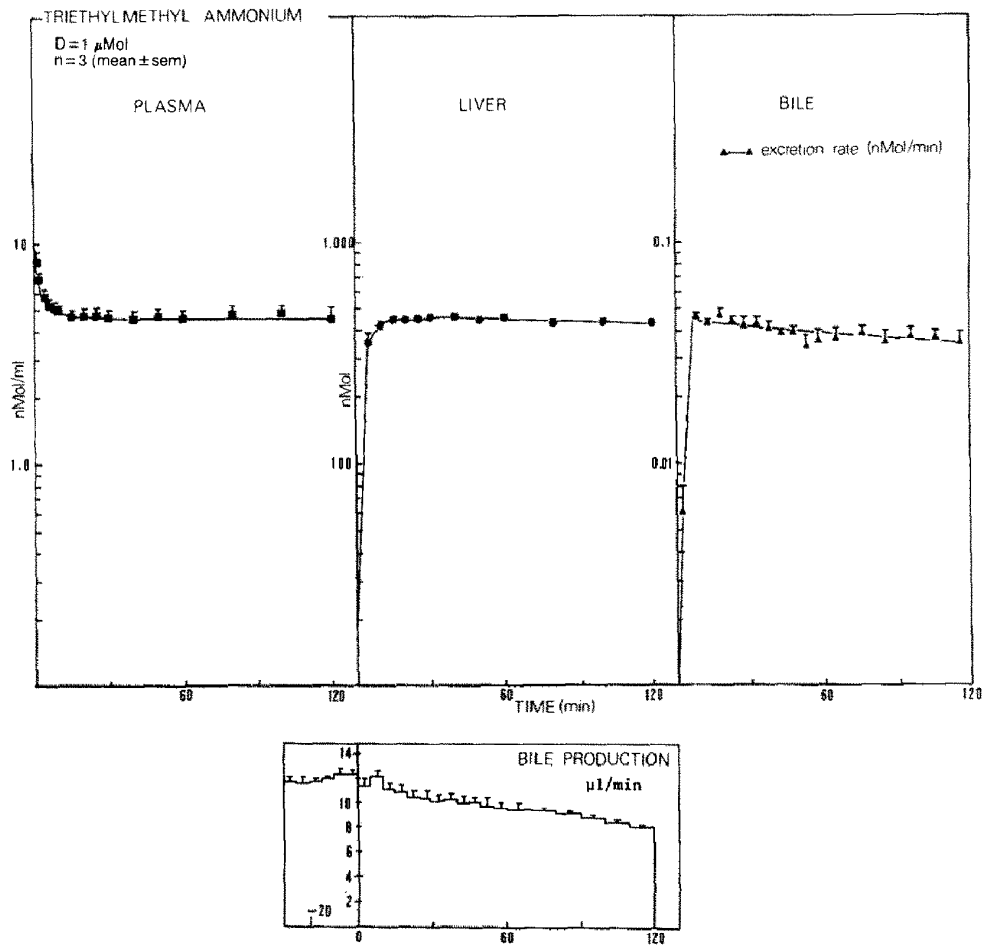

Fig. 4. TEMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. Details are as indicated in the legend of Fig. 1.

Concentration ratios. For all experiments the ratios of the concentrations in perfusion medium (P), liver (L) and bile (B) were calculated (Table 2). All three compounds accumulated in the liver (liver-to-plasma ratio > 1). L/P ratios were 9, 16 and 30 for TEMA, TPMA and TBuMA respectively for the control experiments (D = 1 μmole). An increased liver content (higher L/P ratios) was observed for TEMA if Tc was infused. However, Tc infusion did not change the L/P ratio for TPMA or TBuMA. In contrast to the L/P ratio the B/L ratio of the QACs showed largely different values. Control values were 0.1, 1.3 and 14 for TEMA, TPMA and TBuMA. Tc clearly decreased this ratio for TEMA. But the B/L ratio of TPMA and TBuMA was practically unaffected. From the B/P ratio it can be seen that TEMA was not concentrated in the bile, in spite of major accumulation in the liver. TPMA and TBuMA reached concentrations in bile considerably exceeding that of plasma.

DISCUSSION

General kinetic considerations

Passage of drugs across biological membranes generally can be divided into two categories of processes: passive transfer and specialized transport [22, 23]. Passive transfer can be simple transmembrane diffusion, in which the rate of transfer is directly proportional to the concentration gradient across the membrane. This concept includes possible filtration: transport of a solute in water across a porous membrane. Facilitated diffusion mediated by membrane carrier-molecules and pinocytosis or endocytosis are considered to be specialized transport processes. Carrier transport can be distinguished in primary or secondary active transport or facilitated diffusion. Active transport is considered to be a process which can transport the solute against an electrochemical gradient. Such an electrochemical gradient may be expressed by rearrangement of the Nernst equation

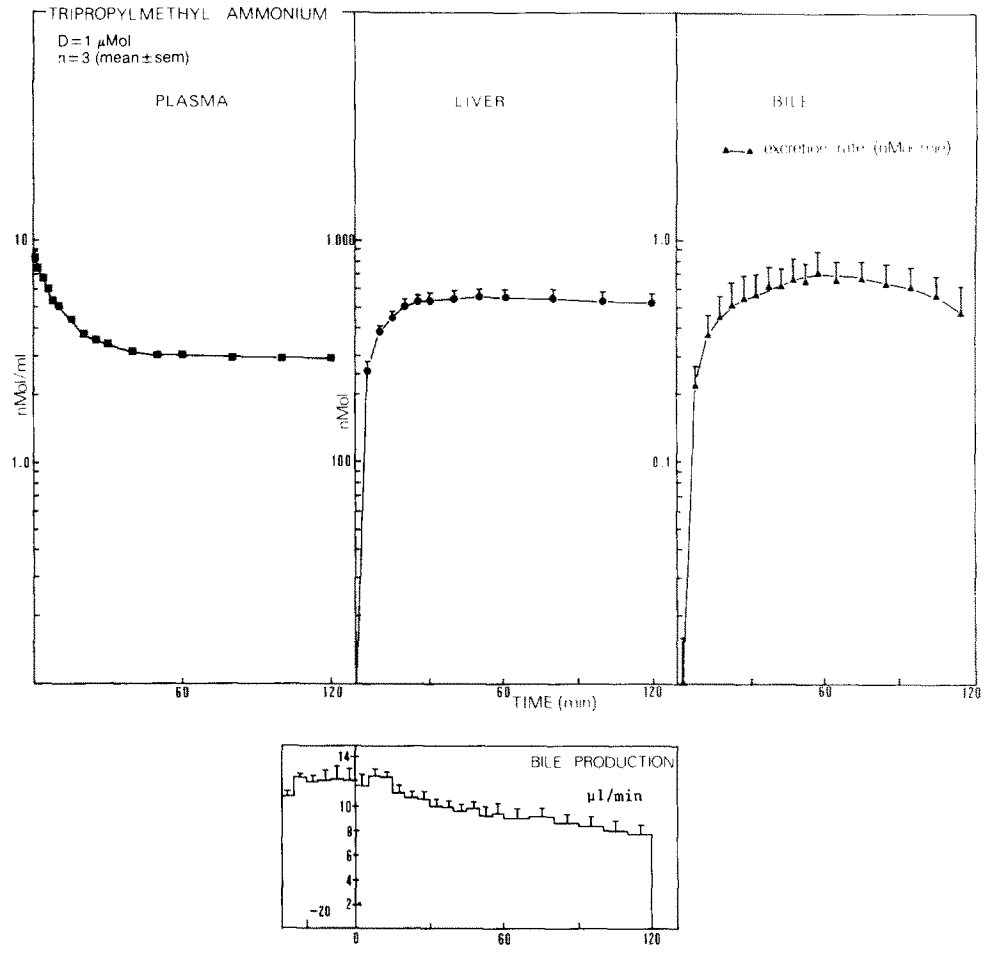

Fig. 5. TPMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. Details are as indicated in the legend of Fig. 1.

$$\frac{C_1}{C_2} = e^{-ZFV_m/RT}$$

where C_1 and C_2 are the concentrations on both sides of the membrane (C_1 = inside, C_2 = outside), Z, F, R and T are the usual constants and V_m is the transmembrane electrical potential (mV). At 37° this relationship is

$$\frac{C_1}{C_2} = e^{-0.0374 \times V_m}$$

Several studies described the hepatic transport processes for quaternary ammonium compounds (for reviews see refs. 3 and 4).

Our experiments were performed with compounds of low (TEMA), moderate (TPMA) and relatively high (TBuMA) lipophilicity. Partition coefficients in n-octanol/Krebs buffer were 0.0013, 0.013 and 0.14 respectively, increasing approximately by a factor of 10.

To detect possible dose dependent kinetics two different doses were studied: 10 and 1 μmole. Only slight differences between these doses were found for TEMA, whereas the hepatic uptake rate and the biliary output of TPMA and TBuMA were clearly higher at the lower dose. The concentration profiles at this dose could well be fitted according to a first order two compartment model. The clearance values found at D = 1 μmole equalled the previously estimated hepatic clearance values *in vivo* in the rat, studies in which a dose of 4 μmoles of the particular QACs were given [14]. This confirms the proper preservation of transport functions of the isolated perfused liver as reported before [7].

On the basis of the kinetic profiles both *in vitro* and *in vivo* the dose of 1 μmole was assumed to provide first order kinetic conditions in these transport studies.

At this dose it is of interest to compare the hepatic handling of the three compounds. The following characteristics can be mentioned.

All three compounds are rapidly taken up into the liver and after the initial distribution phase reach liver-to-plasma ratios of 9, 16 and 30 respectively (Table 2). Initial disappearance half lives were 1.1, 5.3 and 4.6 min (Table 1), while the rate constants

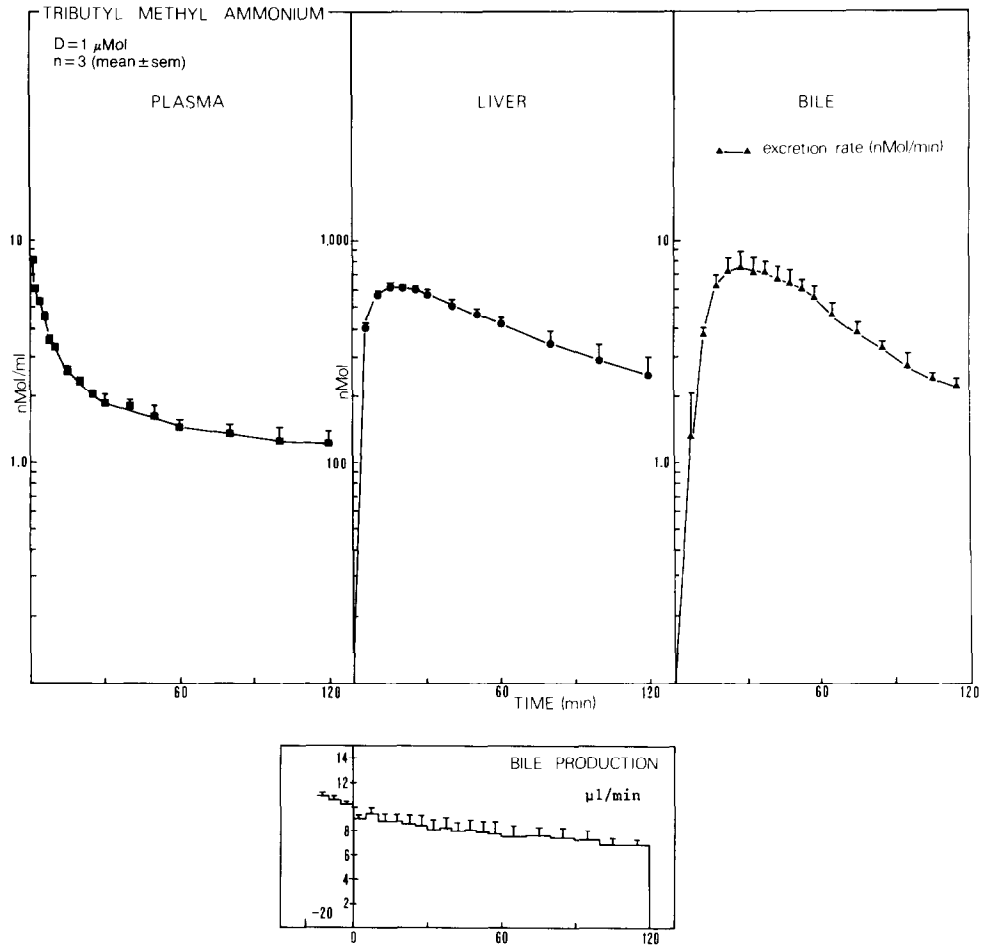

Fig. 6. TBuMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. Details are as indicated in the legend of Fig. 1.

for hepatic uptake (k_{12}) amounted to 0.312, 0.077 and 0.110 respectively (Table 3). Uptake rate for TEMA therefore is at least a factor of three higher than those of the propyl and butyl derivatives. The initial clearance of TEMA ($k_{12} \times V_1$) is about 36 ml/min and approaches the liver perfusion flow. Clearance of TEMA without flow limitation would therefore be much higher. For this compound apparently very rapid membrane transport occurs, which may be carrier mediated in spite of the fact that in contrast to TPMA and TBuMA no distinct saturation of this process occurred within the dose range studied.

Possibly the K_m of the particular sinusoidal membrane transport is relatively high for this compound (low carrier affinity). The liver-to-plasma concentration ratios of the compounds ranging from 10 to 30, at first sight considerably exceed the value which can be anticipated if passive equilibration according to the membrane potential would occur. This ratio would be 3.7 if the liver-to-plasma membrane potential is assumed to be 35 mV (negative inside the cells). Ratios of 10–30 would therefore imply active transport if the assumption is correct that little intracellular binding to cytosolic macromolecules and cell organelles occurs and also that total liver concentration mainly reflects drugs present in hepatocytes. Data on the subcellular distribution of TEMA, TPMA and TBuMA according to [20] indicated that the agents are present in the liver cytosol fraction in the unbound form. This is in agreement with the extremely low binding to plasma proteins. If corrections were made for binding to the particulate fractions (membranes and cell organelles) the unbound cytosol/plasma concentration ratio was calculated to be 14, 12 and 33 respectively. These findings imply that unbound cytosol/plasma ratios of TPMA and TBuMA are in the same order as the L/P ratio depicted in Table 2, whereas the L/P ratio of TEMA is 60% lower and the B/L ratio corresponding higher than the calculated cytosol/plasma ratios. In earlier studies from our laboratory with PAEB and N^4-acetyl-PAEB [20] it was also calculated that the concentration ratio of the unbound compounds between liver cytosol and plasma exceeded a factor of 10. Accumulation in the liver, however, is not only due to plasma-to-liver membrane transport and

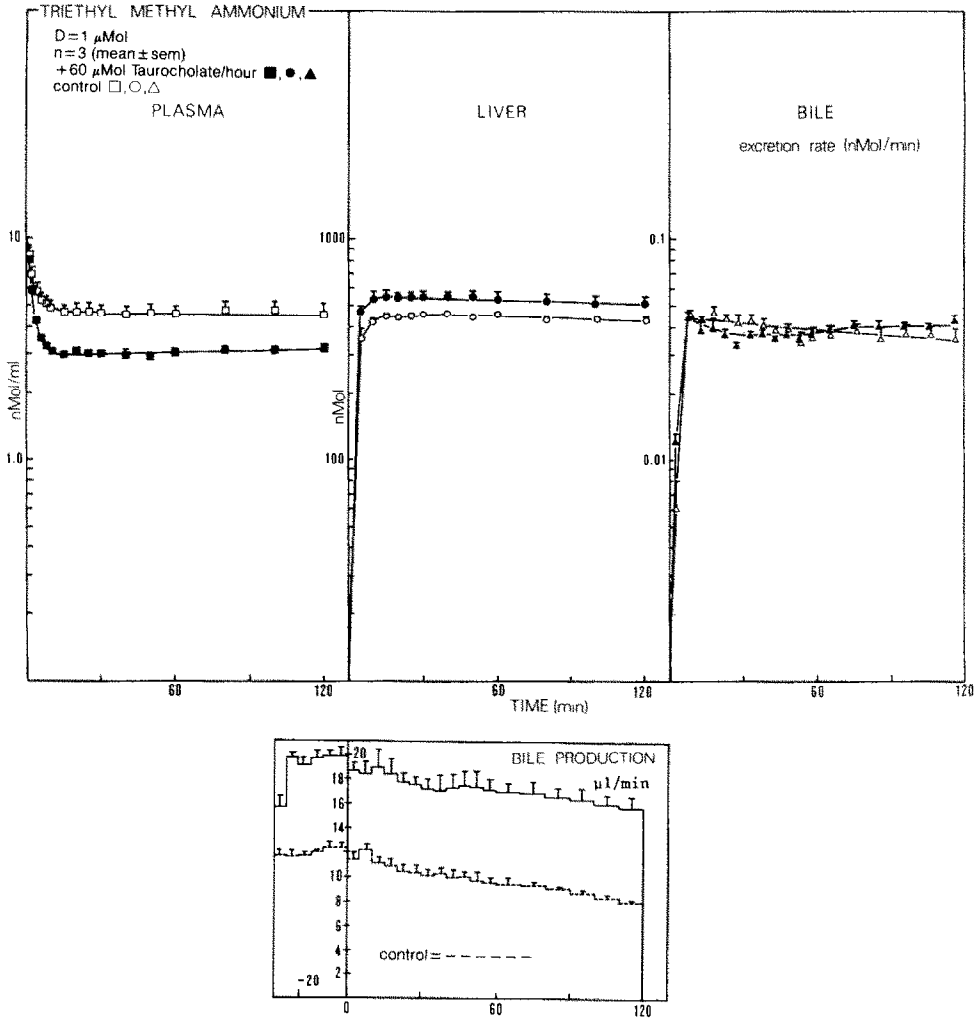

Fig. 7. TEMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. A taurocholate (Tc) infusion of 60 μmoles/hr is given. Control values for D = 1 μmole are depicted (□, ○, △). Further details are as indicated in the legend of Fig. 1.

subsequent sequestration but it is also dependent on the rate of liver-to-plasma transport. The kinetic pattern for the three compounds strongly suggest bi-directional transport between plasma and liver. The rate constants (k_{21}) for liver-to-plasma transport decrease going from TEMA to TBuMA (Table 3). It is possible that liver-to-plasma membrane transport decreases with increasing lipophilicity, although an increased cellular binding (increase in liver distribution volume) is certainly not excluded. Anyway, liver-to-plasma concentration ratio of TEMA, TPMA and TBuMA roughly increase with a factor of 2 between these compounds while partition coefficients increase with a factor of 10.

Comparing the uptake rates of TPMA and TBuMA (rate constants and initial half lives, Table 1 and 3), these values indicate that the propyl derivative is relatively slowly taken up into the liver. Uptake into the liver even may be rate limiting for the overall hepato-biliary transport (see liver content and biliary excretion curves, Figs. 2 and 3), a condition which was suggested earlier for acetylated PAEB [5, 24]. In this respect it is of interest to note that the biliary clearance of TPMA (calculated from excretion rate and plasma concentration, $(dAe/dt)/C_p$, in the terminal phase of the disappearance curve, being about 0.3 ml/min) is significantly lower than the clearance calculated by D/AUC, which is 0.8 ml/min and in accordance with our *in vivo* studies [14]. This can be explained by slow equilibration between plasma and liver limiting the intracellular concentration (driving force) determining the biliary output [25]. Another possibility is that in addition to biliary clearance irreversible binding to cell organelles such as lysosomes takes place, which can be considered as another (intrahepatic) clearance process. Evidence for such a binding was obtained earlier from subfractionation studies [20, 26–29], but also using autoradiographic [30], electronmicroscopic [27] and pharmacokinetic analysis [5]. How-

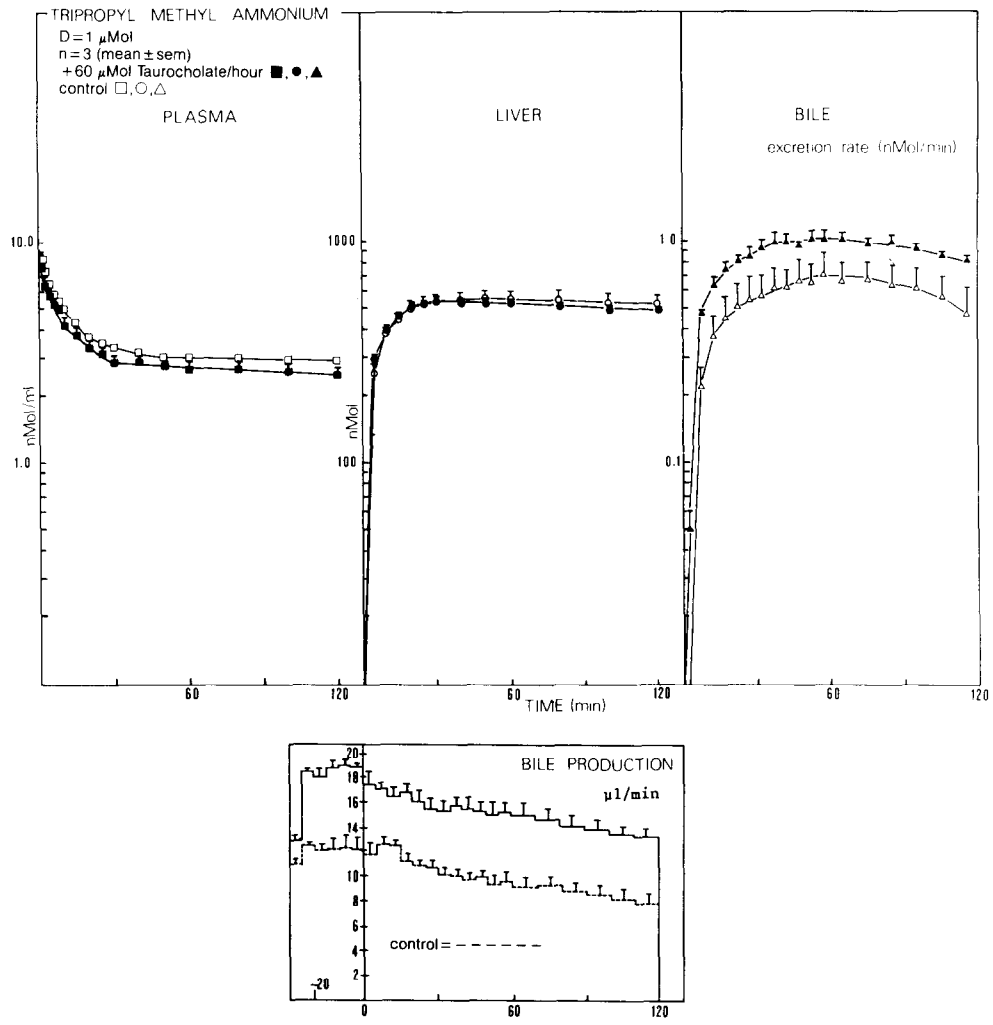

Fig. 8. TPMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. A taurocholate (Tc) infusion of 60 μmoles/hr is given. Control values for D = 1 μmole are depicted (□, ○, △). Further details are as indicated in the legend of Fig. 1.

Table 3. Rate constants (mean values) for transport from the central into the peripheral compartment (k_{12}) and from the peripheral into the central compartment (k_{21}) calculated according to an open two compartment model with elimination from the central (k_{10}) or from the peripheral (k_{20}) compartment

Compound	Dose	k_{12} (min^{-1})	k_{21} (min^{-1})	k_{10} (min^{-1})	k_{20} (min^{-1})
TEMA	1	0.31	0.32	0.0057	
+Tc	1	0.41	0.18*	0.0094*	
TPMA	1	0.077	0.051		0.0049
+Tc	1	0.085	0.055		0.0048
TBuMA	1	0.11	0.036		0.0067
+Tc	1	0.092	0.017*		0.0069

Standard errors of the mean were less than ±20%. Data are given for the dose D = 1 μmole. The influence of sodium taurocholate (Tc) is shown.

* Statistically significant differences with controls ($P < 0.05$).

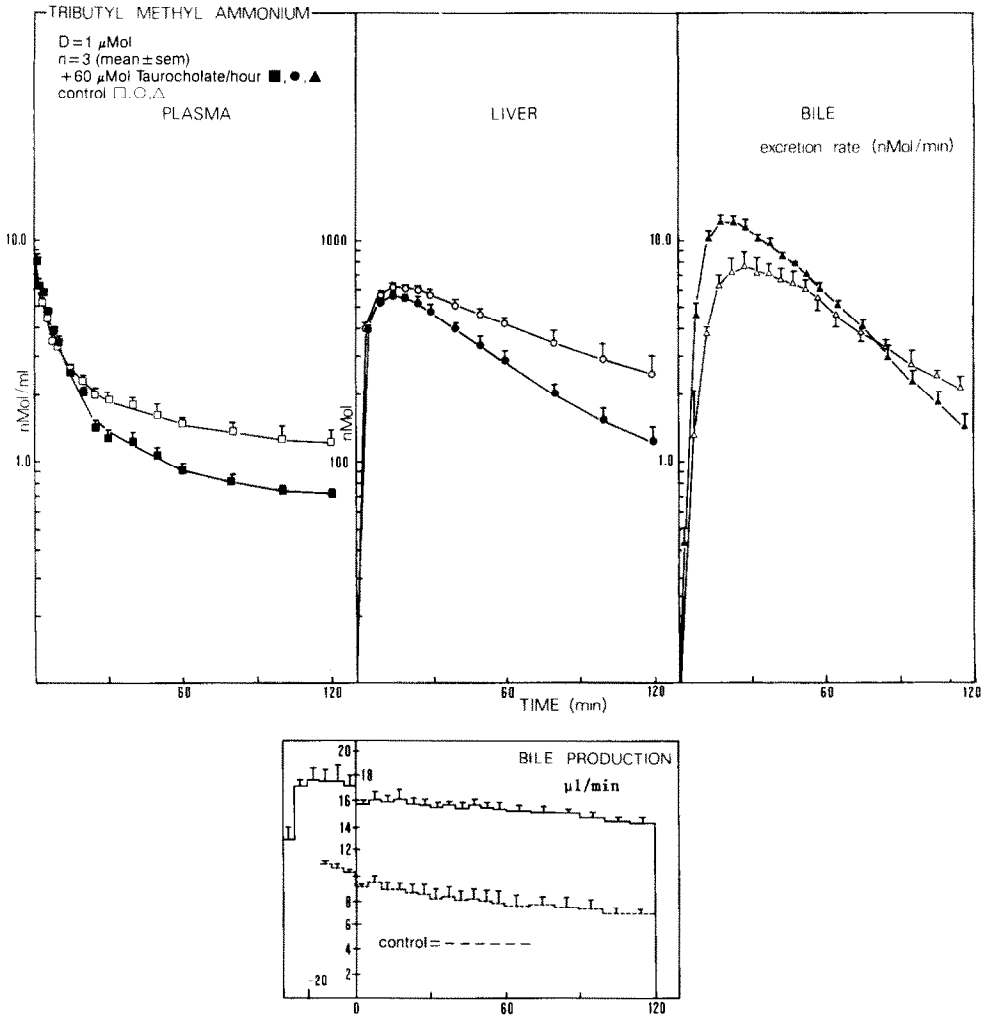

Fig. 9. TBuMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. A taurocholate (Tc) infusion of 60 μmoles/hr is given. Control values for D = 1 μmole are depicted (□, ○, △). Further details are as indicated in the legend of Fig. 1.

ever, a similar discrepancy between biliary clearance and plasma clearance was not observed for TBuMA which is excreted into bile much more efficiently. In contrast to Ruifrok's studies with isolated membrane vesicles from the liver [10] we observed efficient transport of QACs with low lipophilicity. This may indicate loss of carrier function or normal permeation characteristics in the vesicles or an indication for abnormal orientation or a non-sinusoidal origin of the membrane preparation.

Biliary excretion patterns for the three agents varied even more than was observed for hepatic uptake. Biliary output was 0.5, 6.6 and 52.1% of the injected dose during 2 hr perfusion respectively. Bile-to-liver ratios were 0.1, 1.3 and 14 respectively (Table 2). It is striking that these parameters increase approximately with a factor of 10, very much in line with the difference in partition coefficients. Biliary transport therefore seems to be directly correlated with lipophilicity of the QACs. The fact that the k_{20} values for liver-to-bile transport of TPMA and TBuMA (indicating the fraction of the drug present in the liver which is transported into bile per unit of time) differed only slightly (Table 3) can be explained by the much greater liver distribution value for TBuMA.

The quantitatively unimportant biliary excretion of TEMA can in principle be explained by passive processes: the bile/plasma concentration ratio was very close to 1, this in spite of accumulation in the liver (bile/liver ratio was only 0.1). The lack of efficiency for net canalicular transport could be explained by a low affinity for the canalicular carrier combined with reabsorption from the bile into the cells, driven by the negative membrane potential. However, it is also possible that the small molecular weight compound enters bile directly from the plasma via the pores in the tight junctional complexes (paracellular transport). Evidence for reabsorption from the biliary tree has been reported by Back and

Calvey [31] in studies with the drug edrophonium. Bradley [32] concluded from studies with inert, pair matched solutes of various charge and size that negatively charged pores large enough to permit passage of compounds (MV up to 342 Daltons, radius up to 102 Å) are present between the bile and plasma compartments. As discussed later on, taurocholate increased uptake of TEMA in the liver, but did not increase the biliary output, an observation which is in line with such a paracellular transport.

The bile/liver ratio of TPMA was a factor of 10 higher than that of TEMA. The ratio of one may suggest passive canalicular transport for this compound. However, it should be stressed that in spite of this ratio, canalicular transport occurred against an electrical gradient and active transport is not excluded.

Bile/liver ratio of TBuMA was even much higher and if binding to biliary micelles would be moderate as found for PAEB [5, 20, 33], this would certainly imply active carrier mediated transport.

Influence of taurocholate

Figures 7–9 show that taurocholate affected hepato-biliary transport of the three model compounds. Two effects can be directly read from the figures and the calculated concentration gradients in Table 2.

1. Biliary output of TPMA and TBuMA clearly increased in contrast to that of TEMA, whereas the bile/liver concentration ratios were not appreciably changed for the three cations.

2. Liver content relative to the plasma concentration is clearly increased for TEMA, but not significantly in the case of TPMA and TBuMA.

Taurocholate may in principle have two effects: increase of net uptake into the liver and increase in the rate of excretion from the liver. Since biliary output of TEMA is negligible the increased liver/plasma ratio is clearly expressed, whereas the two effects compensate each other in the case of TPMA and TBuMA, resulting in similar liver/plasma ratios. Taurocholate should be unable to increase the uptake of TEMA, since this is already maximal, i.e. limited by flow. It may, however, reduce reflux from the liver. This is indeed suggested by the lower k_{21} value (Table 3), as compared to the control.

Taurocholate can form ion pair complexes with onium compounds [15, 34, 35], a phenomenon which was confirmed by us for the model compounds under study [36]. Since taurocholate is also transported into the hepatocytes, intracellular ion pair formation may influence the intracellular distribution or binding of the organic cations. The more lipophilic ion pairs may more easily associate with macromolecules, which pharmacokinetically implies an increase in distribution volume. This would explain the lower k_{21} value, at an uncharged membrane transport (Cl_{21}). Transport of TEMA into bile could be unaffected, since as discussed above, it may occur directly from plasma. However, lowering of the plasma concentration by Tc (Figs. 7–9) did not result in a lower biliary output. Probably such an effect is compensated for by the increased bile flow, which can be viewed as a filtration process. If no restriction in pore transport occurred due to size or charge of the molecules, biliary clearance and bile flow should increase proportionally. This was indeed observed for TEMA.

The increase in bile flow also elevated biliary clearance of TPMA. Since biliary output increased only from 6.6% to 10.3% of the dose, only slight changes in plasma concentration and liver content can be anticipated (see Fig. 8). k_{12} was somewhat higher, but k_{21} unchanged in contrast to a clear decrease in the latter parameter observed with TEMA and TBuMA. The reason for the increased biliary output of TPMA is yet unclear. If reabsorption from bile into the cells occurred the higher bile flow *per se* may explain this effect. In this respect it should be emphasized that biliary clearance did not increase proportionally with the bile flow.

In the case of the most lipophilic compound TBuMA, the effect of taurocholate on biliary clearance was very marked (Fig. 9). Since biliary output of this agent is high, the increased biliary clearance (proportional to bile production) resulted in a distinct lowering of the plasma concentration. The liver content was identical to controls initially, but thereafter declined much faster. k_{12} was basically unchanged, but k_{21} was clearly lower than in controls. The liver-to-bile fractional transport rate constant (k_{20}) was unchanged, which can be explained by an increased Cl_{20} (net canalicular membrane transport) combined with an increased liver distribution volume ($k_{20} = Cl_{20}/V_2$). The latter effects could be related to the ion pair formation of the QAC and Tc, leading to an increased intracellular binding. The increased bile flow induced by taurocholate may lower bile-to-liver transport and thereby could increase net canalicular transport. Alternatively binding to biliary micelles may play a role in such a reduced reabsorption and also may explain the dissimilar effects of taurocholate on the kinetics of the three agents of different lipophilicity.

The present results confirm earlier studies of MacGregor and Clarkson [16] and Kuo and Johnson [17] reporting stimulatory effects of bile salts on hepato-biliary transport of QACs. The lack of this effect in other studies [5, 15] is probably due to the fact that uptake into the liver rather than canalicular transport of the compounds studied was the rate limiting step in the overall plasma-to-bile transport. Such a phenomenon may also explain the small effect of Tc (Table 1) on the biliary clearance of TPMA in the present study. In conclusion: the three organic cations studied exhibit markedly different transport patterns and do not appreciably bind to plasma and liver cytosol proteins. They may provide very useful tools to study hepato-biliary transport function for various classes of organic cations.

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