*Biochemical Pharmacology*, Vol. 33, No. 24, pp. 3991–4002, 1984.
Printed in Great Britain.

0006–2952/84 $3.00 + 0.00


# HEPATIC UPTAKE AND BILIARY EXCRETION OF ORGANIC CATIONS—II

## THE INFLUENCE OF ION PAIR FORMATION

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(*Received* 4 *November* 1983; *accepted* 9 *July* 1984)

**Abstract**—The influence of an anorganic anion iodide ($I^-$) and an organic anion tetraphenylborate ($TPB^-$) on the hepatic uptake and biliary excretion of three organic cations, triethylmethyl ammonium (TEMA), tripropylmethyl ammonium (TPMA) and tri-*n*-butylmethyl ammonium (TBuMA) was studied. The compounds were injected as a bolus (D = 1 μmole) and studied in isolated perfused livers. In the perfusion medium 25% of the amount of NaCl (3 mmole) was replaced by NaI, whereas in two other experiments $TPB^-$ was added to the medium in two concentrations (2 μM and 200 μM).

NaI did not affect the biliary output of the three quaternary ammonium compounds (QACs) although an increased net rate of hepatic uptake was found for all compounds, most likely due to a decreased liver to plasma transport. Liver to plasma concentration ratios were increased, while the ratios between bile to liver and bile to plasma were not affected.

$TPB^-$ in catalytic amounts added to the medium (2 μM) decreased the biliary output of TEMA and TBuMA, whereas the kinetic profile of TPMA was unchanged. The decreased biliary excretion rate of TEMA was explained by a decreased plasma level (due to the increased liver uptake) assuming that the small molecular weight compounds can enter the bile directly from plasma via the junctional complexes between the cells. The bile to plasma (B/P) ratio was not affected. In contrast, the bile to plasma (B/P) ratio and the bile to liver (B/L) ratio of TBuMA were decreased, compared with the control, probably due to an increased reabsorption from the bile, whereas the back transport from the liver into the plasma was also decreased.

A large amount of $TPB^-$ (200 μM), added to the perfusion medium, dramatically changed the kinetic profile of the three QACs. Ion pair formation between the QACs and $TPB^-$ was supposed to be responsible for this effect. Plasma levels dropped more rapidly as a result of an increased rate of liver uptake. The biliary excretion of all compounds was greatly reduced (the excretion rates were 0.022, 0.19 and 0.18 nmole/min, compared with 0.047, 0.71 and 7.5 nmole/min for the controls).

It is concluded that ion pair formation may play a role in the hepatobiliary transport. The rate of liver uptake of the QACs is enhanced in the presence of an anion, which is due to an increase in plasma to liver transport ($k_{12}$) and a reduced liver to plasma transport ($k_{21}$). The increased net uptake into the hepatocytes however does not result in an increased biliary output. In fact the effects of ion pair formation on the biliary output can be strongly negative. The intra membrane potential hill, which is supposed to be influenced by $TPB^-$, may be a barrier for liver uptake of QACs with low lipophilicity and also for reabsorption from the biliary tree of QACs with a high lipophilicity. The remarkable decrease in biliary transport of the QACs caused by $TPB^-$ may therefore be explained by reduction of the effective concentration for the canalicular transport of onium compounds within the cells, due to ion pair formation and/or an increased intracellular binding or alternatively to an increased bile to liver transport (increased biliary reabsorption).

Hepatic transport and biliary excretion of organic cations like quaternary ammonium compounds (QACs) have been reviewed by Schanker [1], Smith [2] and Meijer [3]. Transport studies with such compounds were performed in many studies *in vivo* in intact animals [4], several *in vitro* studies in isolated perfused liver [3], in experiments with liver slices [5, 6], hepatocytes (see [7] for references) and liver membrane vesicles [8]. In a previous study [9] we found three simple aliphatic QACs to be good 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. Triethylmethyl ammonium (TEMA) represents a category of compounds with a low lipophilicity which are excreted into the bile in small amounts (less than 1% of the dose). Biliary clearance of these compounds is equal to the bile flow. Tri-*n*-butylmethyl ammonium (TBuMA) represents a second group of highly lipophilic compounds which are excreted into the bile in large amounts (more than 30% of the administered dose). Tripropylmethyl ammonium (TPMA) belongs to a group of QACs with intermediate lipophilicity. Its biliary excretion ranged between 5 and 15% of the administered dose *in vivo*. Uptake into the liver may be rate limiting in the overall hepatobiliary transport of such compounds [10–12].

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Quaternary ammonium compounds are positively charged in physiological conditions and are able to form ion pairs with large negative anions [13, 14]. Many authors investigated the transfer of such organic ion pairs across body membranes, but in most cases transport across the wall of the gastrointestinal tract was the subject of these studies (for review see [15]). Ruifrok [8] recently studied the uptake of some quaternary ammonium compounds into rat liver plasma membrane vesicles and into rat intestinal brush border membrane vesicles [8, 16]. In these studies only significant transport for cations which possessed a high lipophilicity was observed. Uptake into the liver membrane vesicles showed characteristics of a passive transport process, which was not stimulated by a negative membrane potential, suggesting membrane permeation in an uncharged form. Ruifrok also studied the influence of a catalytic amount of tetraphenylborate ($TPB^-$) on the transport of QACs into rat liver cell vesicles. $TPB^-$ was found to stimulate the rate of uptake of highly lipophilic QACs into the plasma membrane vesicles of the liver. In the presence of $TPB^-$ the uptake was stimulated by a negative diffusion potential, suggesting that small amounts of $TPB^-$ in the membrane facilitate the permeation of the positively charged agent. The mechanism of this effect is not yet elucidated but could be related to masking of positively charged membrane compounds or a role of $TPB^-$ as an artificial carrier for cation translocation.

*In vitro* extraction studies [13, 14, 17] showed that QACs form ion pairs with iodide. These ion pairs are well extracted from aqueous solutions into organic solvents, such as n-octanol. The increase in octanol to water partition of these agents increased to the same extent for the various compounds (Table 1). Hoeberichts [18] found an increased rate of uptake of the lipophilic cation dimethyldibenzyl ammonium (DDA) by yeast cells in the presence of tetraphenylborate ($TPB^-$). This study indicated that DDA was trapped in the cells as a stoichiometric 1 : 1 complex with $TPB^-$. Stark [19] also suggested neutral ion pair formation between $TPB^-$ and positively charged cations to be part of a transport carrier mechanism for positive ions through biological membranes.

In a preceding study [10] we found that by infusion of sodium taurocholate (Tc) an increased biliary clearance of TEMA, TPMA and TBuMA could be observed. Taurocholate also enhanced the net uptake into the liver (increasing the hepatic distribution volume of the QACs). Ion pair formation between the QAC and Tc was proposed as a possible mechanism to explain the latter effects.

Table 1. Partition coefficients of three quaternary ammonium compounds TEMA, TPMA and TBuMA, in Krebs solution ($P_K$), with 0.4 mol iodide in saline ($P_I$), with 20 μmol of $TPB^-$ in saline ($P_{TPB}$), and with 200 μmol of taurocholate in saline ($P_T$)

| | $P_K$ | $P_I$ | $P_{TPB}$ | $P_T$ |
|---|---|---|---|---|
| TEMA | 0.0013 | 0.0074 | 0.023 | 0.006 |
| TPMA | 0.0129 | 0.1053 | 0.104 | 0.043 |
| TBuMA | 0.1412 | 1.823 | 1.447 | 0.585 |

In the present study we investigate the influence of such an ion pair formation in more detail with the model compounds TEMA, TPMA and TBuMA replacing 25% of the amount of NaCl in the perfusion medium by NaI. $TPB^-$ (0.2 μmole and 20 μmole/100 ml respectively) was included in the study since it is supposed to decrease the intramembrane potential hill which is due to an uneven charge distribution within the membranes, and lowers the barrier for passage of charged compounds [20]. $TPB^-$ can also form ion pairs with organic cations [19].

## MATERIALS AND METHODS

*Materials.* $^{14}C$-labelled TEMA, TPMA and TBuMA were synthetized as described previously [17]. Albumin was obtained from Organon Technika, Oss, The Netherlands. All other chemicals were from E. Merck (Darmstadt, F.R.G.).

*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 quaternary ammonium compounds used in this study are not metabolized, binding to plasma proteins is negligible.

*Liver perfusion studies.* The perfusion technique used was as described before [21]. 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 [22]. 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 dose of 1 μmole of the three QACs given as a bolus injection and was used as the control experiment. In one set of experiments 3 mmole of NaCl in the medium, representing about 25% of the total amount of NaCl, was replaced by 3 mmole of NaI. The influence of tetraphenylborate ($TPB^-$) was investigated by adding 0.2 μmole and 20 μmole to the perfusion medium (2 μM and 200 μM). Plasma samples were taken at different times and bile was collected after 5- or 10-min intervals. At the end of the experiments the liver was homogenized in a saline solution (the volume was four times the liver weight) and an aliquot 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.

*Pharmacokinetic analysis of the data.* Plasma disappearance-, liver content- and biliary excretion rate vs time curves were fitted with the computer program RUGFIT (A. H. J. Scaf, personal communication) [23]. The choice of the best fitting compartment model was based on the statistical test with a 95% significance level according to the procedures described by Boxenbaum [24]. Concentration ratios between liver and plasma (L/P), bile and liver (B/L) and bile and plasma (B/P) were calculated. The concentration of the QAC in the liver was calculated

from the calculated content at the midpoint of a time interval divided by the liver wet weight. In those cases that the terminal half life was found to be larger than 240 min the $k_{20}$ was calculated by dividing the excretion rate by the concurrent content in the liver, as $k_{20}$ represents the fraction that is excreted of the amount present in the compartment from which elimination takes place.

## RESULTS

### *Influence of iodide*

Replacement of 25% of the total amount of NaCl in the perfusion medium by NaI (3 mmole) had a similar effect on the plasma disappearance as was reported for taurocholate [10] (Figs. 1–3). Lower plasma levels were found for all three cations, although the effect was not significant for TPMA ($P < 0.05$). NaI increased the liver content of TEMA, which amounted to 59% of the dose compared with 43% for the control at the end of the infusion experiments. No difference was found between the liver content of TPMA and TBuMA and their control experiments in the first hour of the perfusion studies. However, in Fig. 3 it is shown that at the end of the experiment the liver content of TBuMA was considerably higher than the control. We interpret this to be due to a decrease in bile flow which occurred in all experiments with $I^-$ after 1 hr of perfusion (± 25% decrease). Although the decrease in bile flow can explain the decreased excretion rate of TBuMA and TPMA after the first hour, the excretion rate of TEMA already dropped 5 min after administration of the compound.

### *Influence of tetraphenylborate* (2 μM)

Addition of a catalytic amount of $TPB^-$ (0.2 μmole into 100 ml medium) to the perfusion medium only affected the plasma disappearance of TEMA significantly (Fig. 4). The shape of the plasma disappearance curve of TPMA differed only slightly from the control curve, which was reflected in the

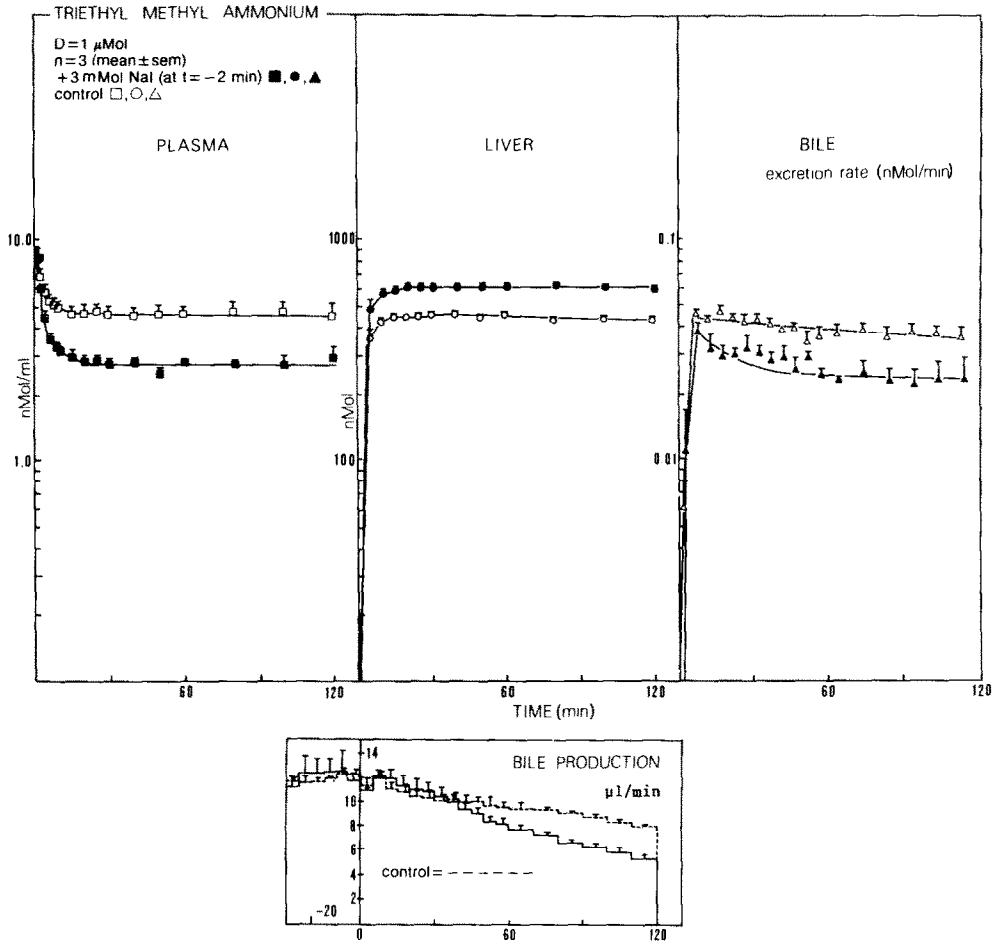


Fig. 1. TEMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. Concentrations in plasma (left), amounts in the liver (middle), biliary excretion rate (right) and bile flow (lower middle) are indicated. The amounts excreted into the bile are expressed as nmole substrate excreted 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. In the perfusion medium 3 mmole of NaCl was replaced by 3 mmole of NaI. Control values are depicted as □, ○, △.

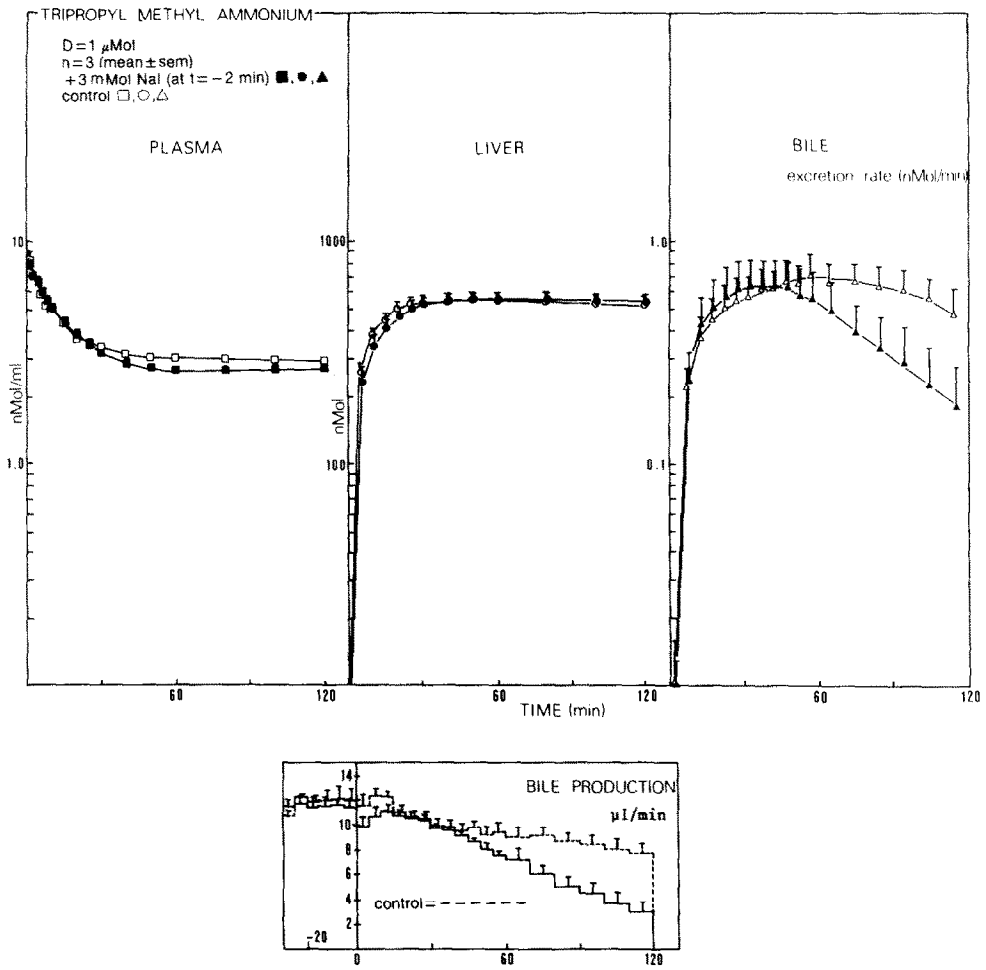


Fig. 2. TPMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. Details are as indicated in the legend of Fig. 1.

distribution half life (6.7 min compared with 5.3 min for the control, Fig. 5). TBuMA showed a more rapid distribution, but a slower elimination phase (4.6 and 138 min for the control and 4.2 and 232 min if $TPB^-$ was given, Fig. 6). For all compounds these plasma disappearance phenomena were reflected in the liver curves: TEMA was accumulated in the liver to a higher extent (54% of the dose, compared with 43% for the control). TPMA uptake in the liver equalled the control value after 2 hr. The liver curves of TBuMA differed only slightly at the end of the experiment where the liver content was about 7% more than in the control experiment. The biliary excretion rate of TPMA was unaffected, whereas the rates of biliary excretion of TEMA and TBuMA were decreased. Note in the figures the different scales for the excretion rate curves.

*Influence of tetraphenylborate* (200 μM)

Addition of 20 μmole of $TPB^-$ to 100 ml of the perfusion medium dramatically changed the plasma, liver and biliary excretion patterns (Figs. 7–9). For all compounds the plasma disappearance was more rapid. The plasma level reached a plateau of 3.3, 1.8 and 1 nmole/ml for TEMA, TPMA and TBuMA respectively, compared with 4.5, 2.9 and 1.2 for the controls. These levels were already reached after 25 min and remained fairly constant during the rest of the experiment. Liver content was increased. TEMA was stored in the liver at a level of 61% of the dose (control value 43%), liver content of TPMA amounted to 66% of the dose and that of TBuMA was 80% of the dose (control values 52% and 32% respectively). Biliary excretion was largely decreased in spite of a normal bile flow. This effect was most dramatic for TBuMA: 52% of the dose was excreted in the control, but only 1.8% when 20 μmole $TPB^-$ was added to the medium. The maximal excretion rate dropped from 7.5 nmole/min to 0.18 nmole/min. For TEMA and TPMA this difference was less pronounced 0.047 nmole/min of TEMA for the control compared with 0.022 nmole/min with $TPB^-$ and for TPMA from 0.71 nmole/min to 0.19 nmole/min.

It should be noted that if the effects of both concentrations of $TPB^-$ (2 and 200 μM) on the kinetic profile of TEMA are compared (Figs. 4 and 7), little difference can be observed, in contrast to the situation with the other two QACs.

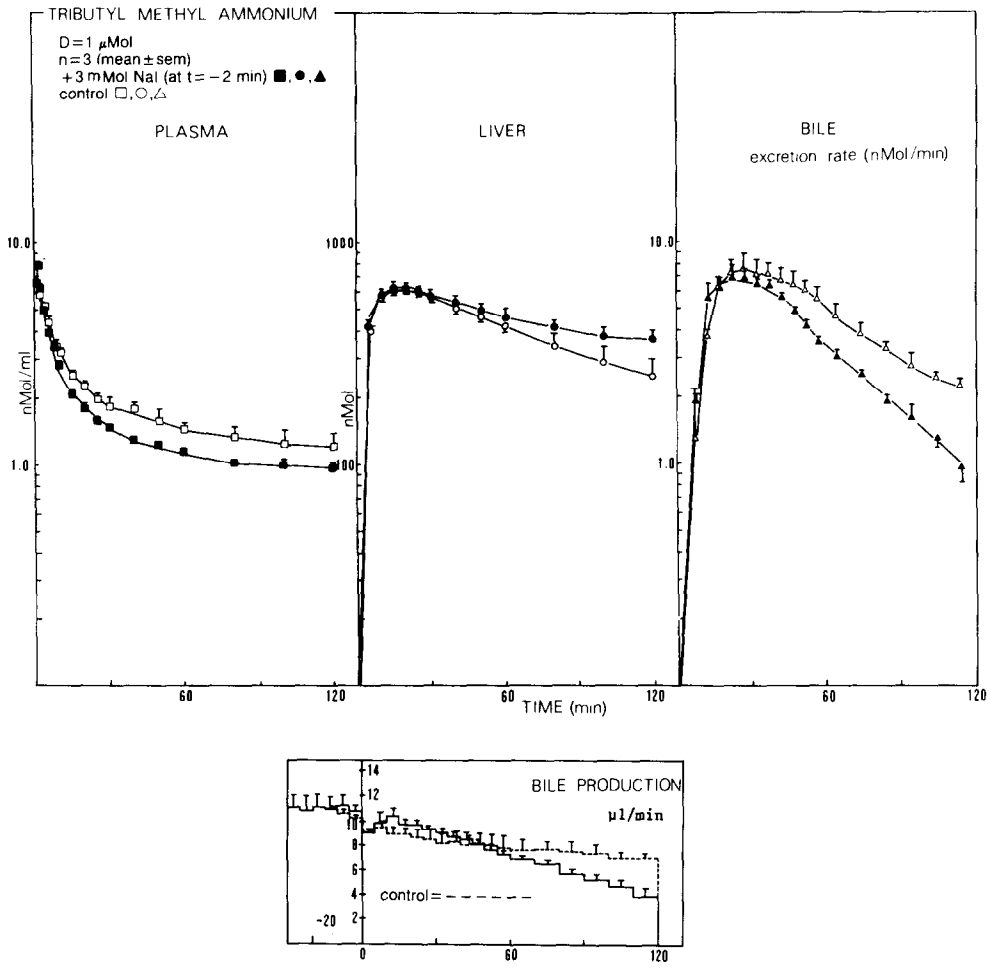


Fig. 3. TBuMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. Further details are as indicated in the legend of Fig. 1.

*Concentration ratios*

For all experiments the ratios of the concentrations in perfusion medium (P), liver (L) and bile (B) were calculated at a time of steady state between liver and plasma (t = 10 min) (Table 2). All three compounds were accumulated in the liver (liver-to-plasma ratio > 1), although not according to their relative lipophilicity. The lipophilicity increased from TEMA to TPMA and from TPMA to TBuMA with a factor of 10. L/P ratios were 9, 16 and 30 for TEMA, TPMA and TBuMA respectively for the control, increasing with a factor of 2. NaI increased the L/P ratio of all three compounds, but this effect was less pronounced for TPMA and TBuMA. Catalytic amounts of $TPB^-$ did not change the L/P ratio significantly for TPMA and TBuMA, whereas the L/P ratio of TEMA was twice the control value. An excess of $TPB^-$ (20 μmole in 100 ml medium) changed the L/P ratio of TEMA with a factor of 2 comparable with the effect of NaI, whereas this ratio increased to three-fold of the control value for TPMA and TBuMA (19, 43 and 93 compared with the control values of 9, 16 and 30 respectively). The B/L ratio was somewhat more affected than the L/P ratio, especially for TBuMA. Control values were 0.1, 1.3 and 14 for TEMA, TPMA and TBuMA respectively. NaI and $TPB^-$ (in both concentrations) decreased this ratio for TEMA with a factor of 2. But the B/L ratio of TPMA was only affected for the high dose of $TPB^-$. NaI

Table 2. Ratios of concentrations in the perfusion medium (P), the liver (L) and the bile (B) during the perfusion experiments at t = 10 min after addition of the drugs (mean values ± S.E.M. are given) (N = 10)

| Compound | L/P | B/L | B/P |
|---|---|---|---|
| TEMA | 9.0/1 | 0.10/0.01 | 0.90/0.04 |
| + NaI | 22/1 | 0.05/0.01 | 1.1/0.1 |
| + $TPB^-$.2 | 17/1 | 0.06/0.01 | 0.9/0.1 |
| + $TPB^-$20 | 19/0.4 | 0.05/0.004 | 0.9/0.1 |
| TPMA | 16/1 | 1.3/0.1 | 21/5 |
| + NaI | 24/2 | 1.0/0.1 | 24/3 |
| + $TPB^-$.2 | 19/2 | 1.3/0.1 | 25/4 |
| + $TPB^-$20 | 43/2 | 0.3/0.04 | 13/2 |
| TBuMA | 30/4 | 14/2 | 404/96 |
| + NaI | 44/4 | 9.2/2 | 400/106 |
| + $TPB^-$.2 | 31/4 | 7.9/1 | 249/55 |
| + $TPB^-$20 | 93/6 | 0.2/0.1 | 21/6 |

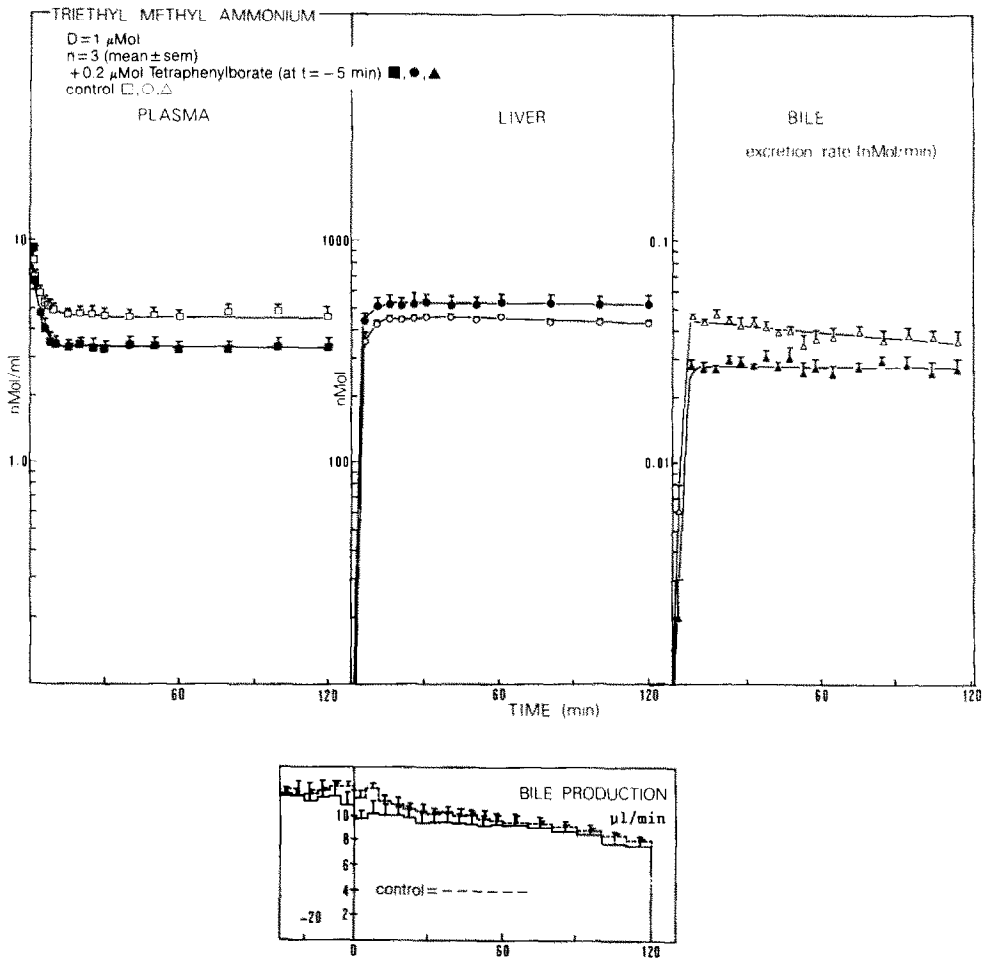


Fig. 4. TEMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. 0.2 μmole of tetraphenylborate ($TPB^-$) was added to the perfusion medium 5 min before the QAC was given. Details are as indicated in the legend of Fig. 1.

decreased the B/L ratio of TBuMA with 30%. Catalytic amounts of $TPB^-$ reduced this ratio for almost half, whereas 20 μmole of $TPB^-$ reduced this ratio to 0.2 (1/70 of the control value).

The B/P ratios of TPMA and TBuMA were lower than the control values only when $TPB^-$ was added in excess, although catalytic amounts of $TPB^-$ also decreased the B/P ratio of TBuMA.

## DISCUSSION

Passive passage of drugs across body membrane (except pore transport) is in principle possible if the drug is electrically neutral and sufficiently lipophilic. In general transfer of charged drugs (anions or cations) is suggested to occur by carrier mediated processes. It is still unclear if this carrier mediated process is electrogenic or occurs electrically neutral (counter transport). Complex-formation of a cation with an anionic cosubstrate and binding to a carrier which recognizes an uncharged species is an alternative possibility. Also non-carrier mediated passive permeation of such a neutral complex can be envisioned. Jonkman [15] discussed ion pair formation and absorption of QACs and recently Ruifrok described the influence of iodide and $TPB^-$ on QAC transport in rat liver membrane vesicles and in intestinal brush border membrane vesicles [8, 16]. Irwin *et al.* [25] were the first to propose a possible role of ion pair formation in facilitating the absorption in the gastro intestinal tract. Later on conflicting evidence was presented for such an effect in several other studies [26–28]. The ion pair hypothesis in drug absorption was rejected by Jonkman [15], who considered ion pair absorption to be more fiction than fact. He pointed out that for sufficient ion pair formation a large excess of the counter ion is required, which can induce aspecific effects on cell membranes. Also even if the ion pair may be more rapidly taken up into the cells, intracellular dissociation of the complex may occur if the counter ion is not accumulated into the cells. Therefore transport out of the cells may limit the effect on trans-mucosal passage. Only in a few studies was the hypothesis of ion pair – membrane transport in the liver [8] and in human red cells [29, 30] systematically tested with

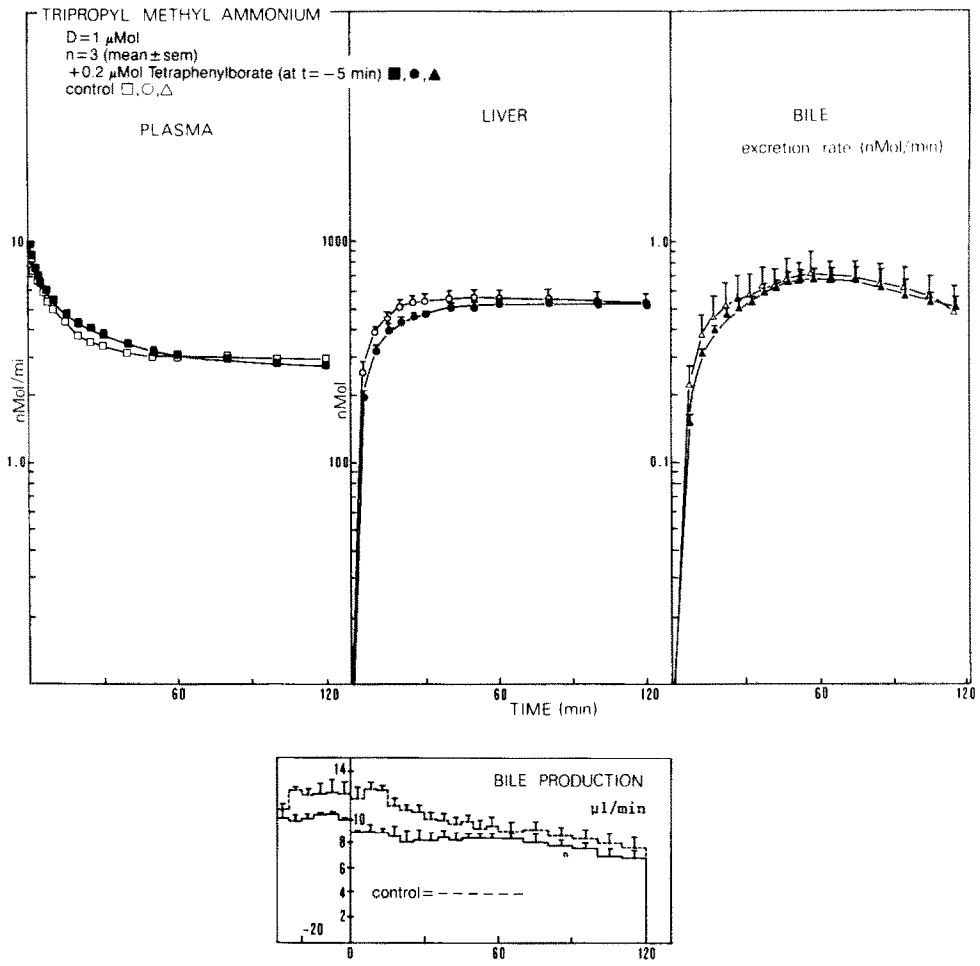


Fig. 5. TPMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. 0.2 μmole of tetraphenylborate ($TPB^-$) was added to the perfusion medium 5 minutes before the QAC was given. Details are as indicated in the legend of Fig. 1.

exogenous counter ions. The possibility of ion pair formation of QACs with endogenous anions such as chloride, phosphate or bicarbonate, however, cannot be neglected knowing that QACs in an octanol–Krebs system gave higher partition ratios than in an octanol–water system [13, 14, 17].

In the preceding study [10] we found that taurocholate (Tc) enhanced the net hepatic uptake of QACs. A decreased transport from the liver into plasma or from the liver into bile could be explained by an increased liver distribution volume. We suggested that ion pair formation between the QAC and Tc might be responsible for this effect, probably by increasing intracellular binding and/or reduction of the effective concentration of the cation, representing the driving force for transport out of the cells. At the sinusoidal membrane ion pair formation may facilitate the passive uptake process, due to an increased lipophilicity of the cation–anion complex. Simultaneously the supposed carrier mediated cation transport would be less effective, as the result of a decreased effective cation concentration. If the latter effect is compensated by the increased passive ion pair transport, the result of both effects could even be a net increase in uptake into the cells. At the canalicular membrane this compensation mechanism may not be present. Since the distribution volume of the QACs is increased by ion pair formation and the effective cation concentration is lowered, a reduction of the transport out of the cells should be anticipated.

*Influence of sodium iodide*

*In vitro* $I^-$ was found to form ion pairs with QACs, which could be extracted into a lipophilic layer (octanol) [13, 14, 17]. Plasma curves and liver-to-plasma concentration ratios indeed demonstrated an increased liver uptake similarly as was found for Tc (Figs. 1–3). These data confirm the suggestion that ion pair formation, resulting in a more lipophilic complex, can play a role in liver uptake transport processes of QACs. The ion pair forming and choleretic compound taurocholate may in principle have three effects: a net increase in the rate of plasma to liver uptake, an increased canalicular transport of the ion pair (that is if the ion pair is really present in the liver) as well as an increased biliary excretion

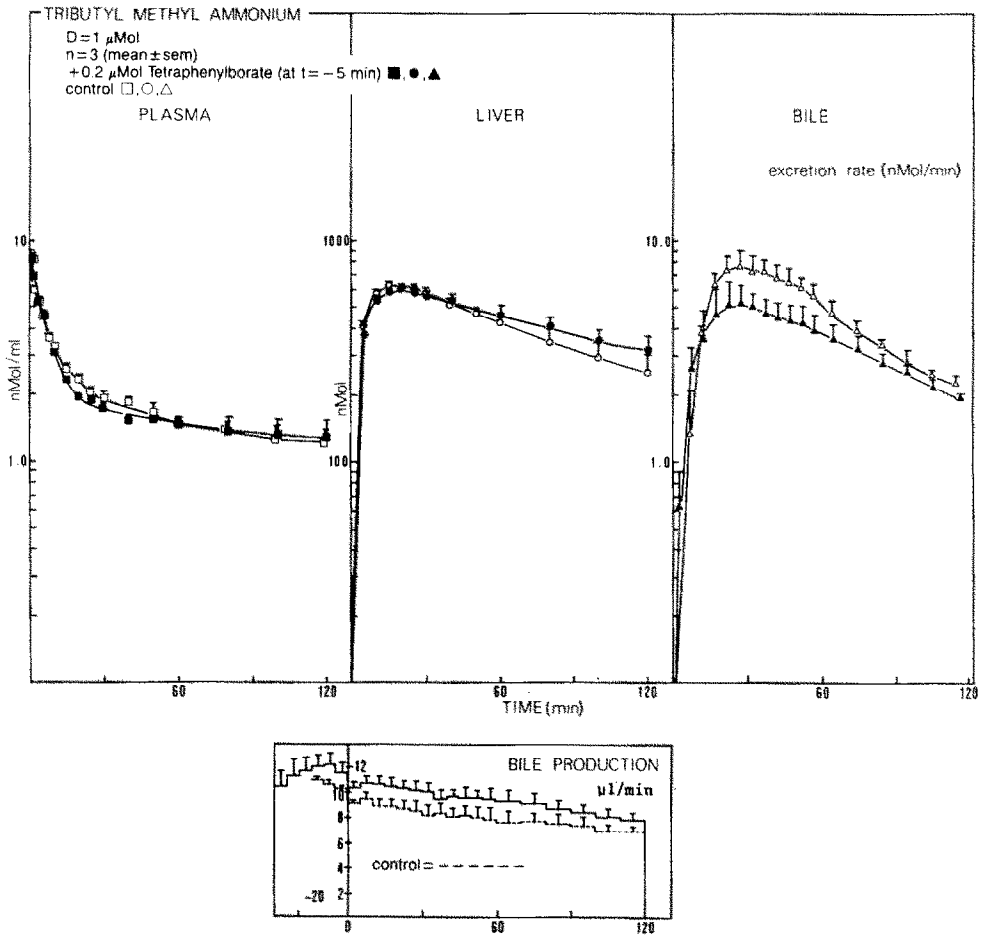


Fig. 6. TBuMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. 0.2 μmole of tetraphenylborate ($TPB^-$) was added to the perfusion medium 5 min before the QAC was given. Details are as indicated in the legend of Fig. 1.

rate, due to the elevated bile flow. The latter two effects may be masked by an increased intracellular binding. In the case of paracellular transport which might occur in the case of TEMA, the excretion rate will be influenced by changes in bile flow and will decrease if ion pair formation enhances distribution of the QAC in the liver. The rate constants of TEMA for liver uptake ($k_{12}$) indicated that the uptake rate was already maximal (plasma flow limited). The increased liver content, induced by $I^-$, was shown to be the result of a decreased back transport ($k_{21}$), whereas the excretion was decreased, probably being the consequence of a lower plasma level. Initially the bile flow was not affected by $I^-$, compared with the control and in contrast to the effect of taurocholate, biliary output of TEMA was clearly reduced. The decreased biliary excretion rate in combination with an increased net hepatic uptake is in accordance with the hypothesis that QACs with a low lipophilicity are transported via the paracellular pathway.

In the case of TPMA (Fig. 2) plasma disappearance, liver uptake, nor biliary excretion seemed to be changed by NaI, although the plasma level was slightly lower than the control value. It is important

Table 3. Rate constants 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 | $k_{12}$ ($min^{-1}$) | $k_{21}$ ($min^{-1}$) | $k_{10}$ ($min^{-1}$) | $k_{20}$ ($min^{-1}$) |
|---|---|---|---|---|
| TEMA | 0.312 | 0.318 | 0.0057 | |
| + NaI | 0.359 | 0.146 | 0.0101 | |
| + $TPB^-$.2 | 0.403 | 0.174 | 0.0097 | |
| + $TPB^-$20 | 0.376 | 0.159 | 0.0098 | |
| TPMA | 0.077 | 0.051 | | 0.0049 |
| + NaI | 0.062 | 0.037 | | 0.0047 |
| + $TPB^-$.2 | 0.063 | 0.039 | | 0.0048 |
| + $TPB^-$20 | 0.139 | 0.035 | | 0.00025* |
| TBuMA | 0.114 | 0.036 | | 0.0067 |
| +NaI | 0.115 | 0.027 | | 0.0054 |
| + $TPB^-$.2 | 0.134 | 0.031 | | 0.0037 |
| + $TPB^-$20 | 0.263 | 0.031 | | 0.00022* |

Data are given for D = 1 μmole. The influence of replacement of 25% of the amount of NaCl by NaI (NaI) and addition of two different amounts of tetraphenylborate ($TPB^-$.2 and $TPB^-$20) is shown. The values indicated by * are calculated from the excretion rate and the amount in the liver.

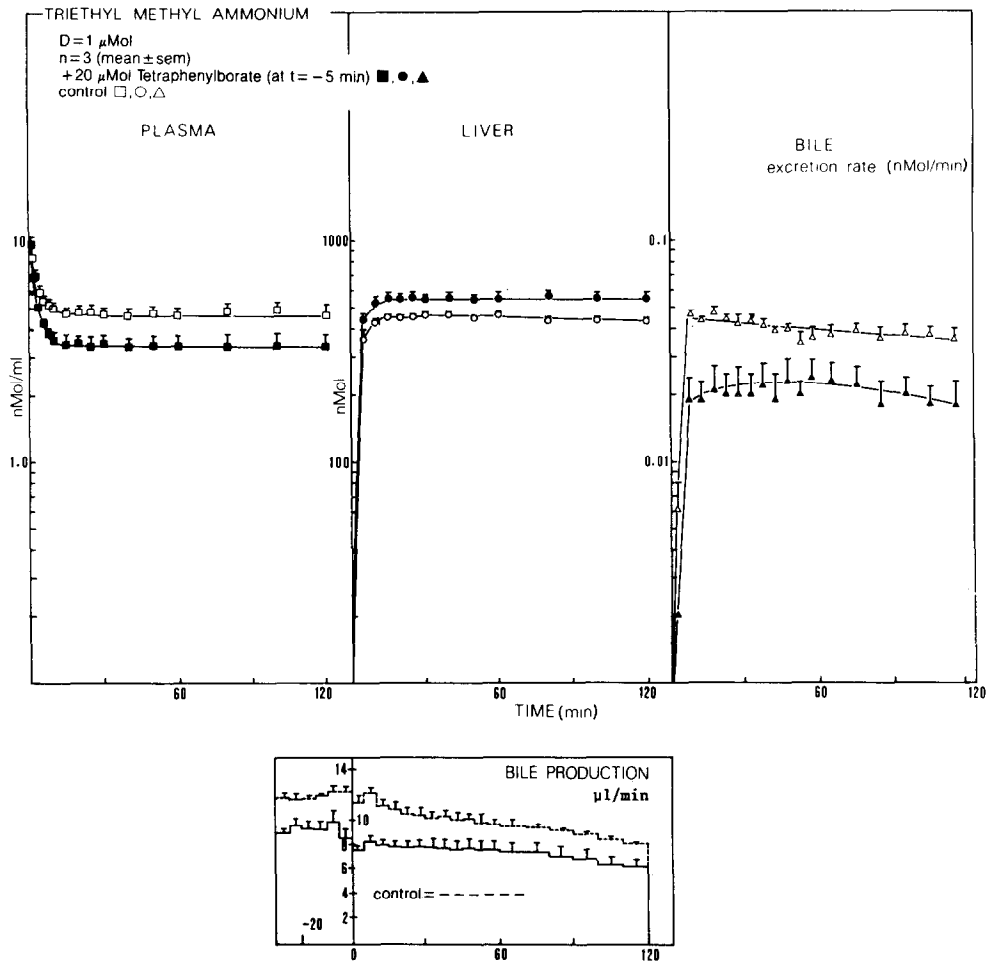


Fig. 7. TEMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. 20 μmole of tetraphenylborate ($TPB^-$) was added to the perfusion medium 5 minutes before the QAC was given. Further details are as indicated in the legend of Fig. 1.

to note that, although hepatic uptake may be the rate limiting step in the overall hepatobiliary transport of TPMA, the presence of $I^-$ did not result in an increased biliary clearance. Since plasma to liver rate constants hardly differed from the controls (Table 3), the decreased $k_{21}$, indicating that back transport from the liver into the plasma was affected, could explain a higher L/P ratio.

More pronounced were the effects of NaI on the kinetic profile of TBuMA. As for TEMA lower plasma levels were found, combined with an increased liver content, whereas in contrast to TEMA the excretion rate initially was unaffected. The faster decrease of the excretion rate after 60 min can be explained by the decreasing bile flow. The rate of liver uptake ($k_{12}$) was unaffected, a higher L/P ratio was probably the result of a decreased back transport ($k_{21}$), whereas the elimination rate constant $k_{20}$ was almost equal to the control value. The overall B/P ratio did not change, indicating that the effect of NaI only concerned liver uptake and storage and in contrast to taurocholate [10] the excretion remained unaffected.

In summary NaI affects the net hepatic uptake of all three QACs under study very likely by decreasing the back transport from the liver into the plasma. The biliary clearance was not changed in the period of a normal bile flow. Ion pair formation between NaI and the QACs might explain these results and support our hypothesis about the dual role of Tc in hepatobiliary transport [10].

*Influence of tetraphenylborate*

Ruifrok [8] found a stimulation of the uptake of QACs with high lipophilicity in liver membrane vesicles by adding catalytic amounts of $TPB^-$ to the medium. Added in catalytic amounts $TPB^-$ was reported to render the transport electrogenic and in the presence of a negative membrane potential would enhance transport of cations across these membranes [20]. In our experiments we found an effect of a catalytic amount of $TPB^-$ (2 μM) on the liver uptake of TEMA, resulting in a lower plasma level and a decreased biliary excretion rate. Similar effects were observed when $TPB^-$ was present in excess, which might imply that also in the high concentration range,

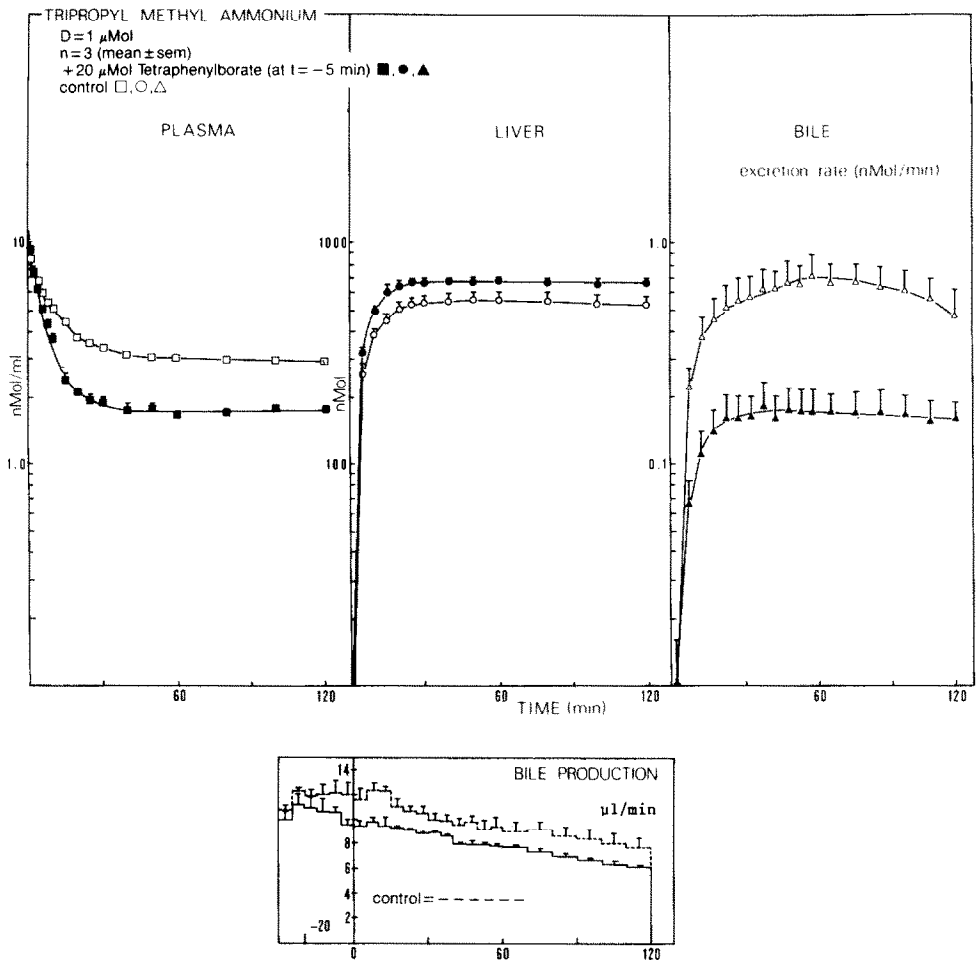


Fig. 8. TPMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. 20 μmole of tetraphenylborate ($TPB^-$) was added to the perfusion medium 5 min before the QAC was given. Further details are as indicated in the legend of Fig. 1.

the $TPB^-$ effect on hepatic transport of TEMA is restricted to the supposed lowering of the intra membrane potential hill and that ion pair formation with this QAC does not play a major role. Plasma and liver curves of TPMA and TBuMA did not differ significantly from the control curves, as was also found for the excretion rate curve of TPMA. A slightly decreased excretion rate was found for TBuMA. Only for TEMA the L/P ratio was increased, whereas for TBuMA the B/L ratio was half the control value. This is in accordance with the decreased excretion rate of TBuMA, which is also demonstrated by a smaller $k_{20}$ (0.0037 $min^{-1}$, compared with 0.0067 $min^{-1}$ for the control). In the case that the intra membrane potential hill is an effective barrier for passive transmembrane transport of cations $TPB^-$ should enhance liver uptake, without decreasing the back transport as was found for Tc and NaI. This indeed is found for TBuMA taking the rate constant $k_{21}$ into account (Table 3). In addition this phenomenon also may occur at the canalicular membrane resulting in a decreased excretion rate constant ($k_{20}$). Possibly reabsorption from the primary bile may be enhanced. a process similar to the improved liver uptake. These phenomena can not be due to ion pair formation, since the concentration $TPB^-$ was much too small to form even 1:1 ion pair complexes.

In contrast higher amounts of $TPB^-$ (20 μmole in 100 ml of the perfusion medium) had major effects as far as TPMA and TBuMA were concerned. The distribution of the two QACs into the liver was much faster, resulting in lower plasma levels, whereas the biliary excretion was almost blocked. The following effects of $TPB^-$ may play a role.

$TPB^-$ is a large negatively charged anion. It may not only lower the intra membrane potential hill, but may also act via an ion pair [19] being the counter ion for the QACs. Ion pair formation (resulting in a more lipophilic complex) might explain the increased liver uptake: in contrast to the iodide and taurocholate anions, both $k_{12}$ and $k_{21}$ were affected by $TPB^-$.

If we assume that $TPB^-$ is also accumulated in the liver, or that $TPB^-$ forms strongs ion pairs, the complex may reduce the effective cation con-

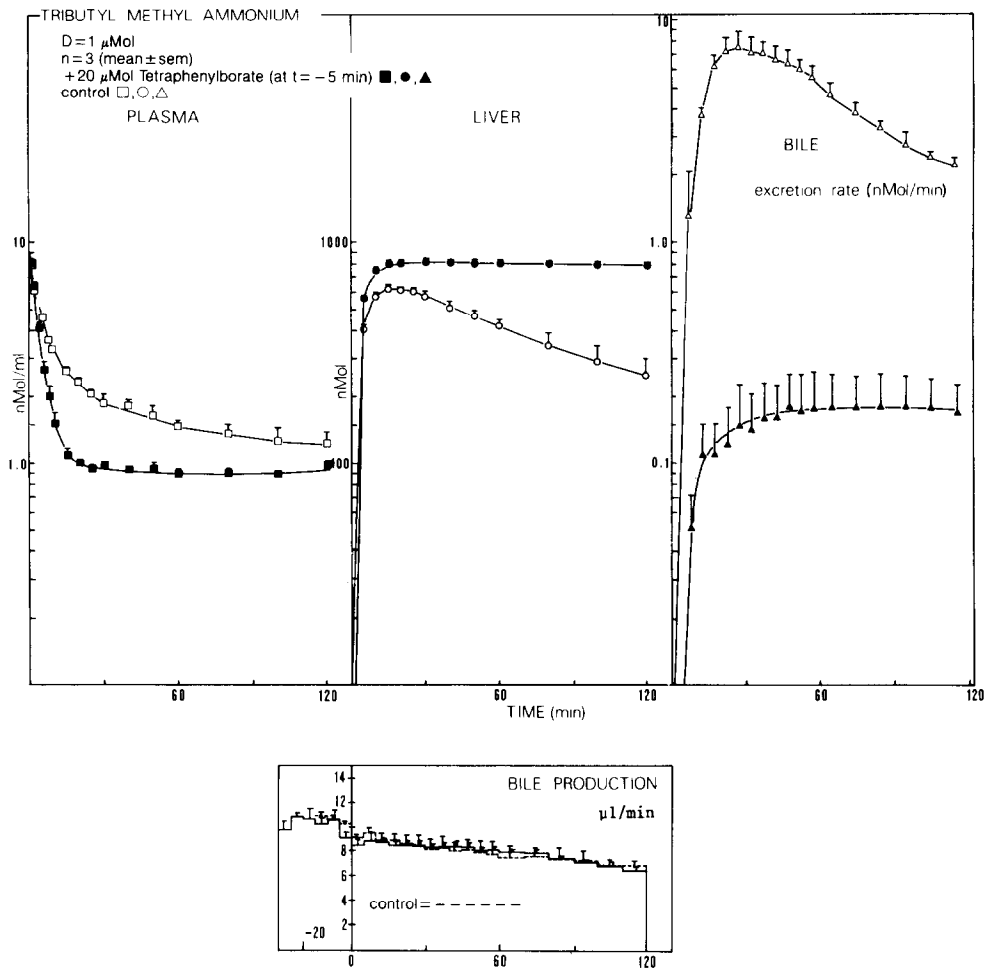


Fig. 9. TBuMA kinetics in the isolated perfused rat liver after a bolus injection of 1 μmole. 20 μmole of tetraphenylborate ($TPB^-$) was added to the perfusion medium 5 min before the QAC was given. Further details are as indicated in the legend of Fig. 1.

centration in the cell and prevent canalicular cation transport, as well as the liver to plasma transport.

The more lipophilic ion-pair might be bound to intracellular macromolecules to a larger extent as the parent QAC, leading to a lower effective concentration of the cation and ion-pair. However, $k_{21}$ was only moderately affected whereas the $k_{20}$ values were more than a factor of 10 decreased.

If $TPB^-$ were concentrated in the liver it could increase the transmembrane electrical potential difference (inside negative). In that case positively charged cations would have an extra barrier to leave the liver via the bile or to return to the plasma. Again it should be noted here that $k_{20}$ was much more decreased than $k_{21}$.

The low biliary excretion of the QACs may be due to a rapid reabsorption of the QACs from the primary canalicular bile, a process which was also proposed for certain organic anions [2]. A highly lipophilic (neutral) ion pair complex, formed by the QAC and the anion $TPB^-$ would be a good substrate for reabsorption, resulting in a lower excretion. Alternatively, in view of the effect of $TPB^-$ on membrane permeation of lipophilic QACs in isolated membrane vesicles, showing that $TPB^-$ induces membrane potential driven cation transport [8], it is quite well possible that $TPB^-$ stimulates cation transport from the canalicular lumen back into the cells, as driven by the negative membrane potential. This would explain the marked decrease in net canalicular transport reflected in $k_{20}$. The mechanism of this peculiar effect of $TPB^-$ is the subject of further studies.

*Acknowledgements*—The authors thank Mr. Roelof Oosting for doing the operations and Prof. Dr. R. A. de Zeeuw for his valuable discussion about the manuscript.

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