Summary

The first part of this thesis deals with the relationship between the chemical structure and pharmacokinetic behaviour of a series of fourteen quaternary ammonium compounds (QAC's) in the rat in vivo. From this series of QAC's subsequently three representative model compounds were chosen for the study of the mechanisms of the hepato-biliary transport in the isolated organ. Furthermore some preliminary transport studies were performed in isolated segments of the small intestine in situ.

Chapter I describes the synthesis of the ^{14}C-labelled QAC's. The iodide salts were prepared from the corresponding tertiary amines with an equimolar amount of ^{14}C-labelled methyliodide. Melting points, octanol-water and octanol-Krebs-solution partition coefficients (log P or log P_{krebs}), the HPLC capacity factor (log k', a parameter indicating the lipophilic-hydrophilic balance) as well as protein binding in plasma were estimated.

The lipophilicity and plasma protein binding (if present) correlated well, irrespective whether partition coefficients were determined in the presence of physiologic concentrations of electrolytes (Krebs-solution), excess of iodide or in simple octanol-water partition systems. These lipophilicity parameters correlated poorly with molecular weight within the whole series, but fairly well within more homologous subgroups in the series of compounds. An attempt was made to determine the predictive value of these parameters with regard to the relative contribution of hepatic, renal, and intestinal excretion to the overall body clearance. This is described in chapter III.

The pharmacokinetic properties were studied in anaesthetized rats after intravenous bolus injection and/or constant infusion. This is described in Chapter II.

Organ distribution and elimination rate into bile, urine and intestinal fluid as well as plasma disappearance were investigated. All compounds showed a biexponential plasma disappearance pattern. Total plasma clearance amounted from 2.3 to 13.7ml/min in general increasing with molecular weight. The relative contribution of biliary, urinary, and intestinal elimination to the total plasma clearance

varied widely within the series of QAC's. Renal clearances exceeded that of mannitol, indicating involvement of active renal transport processes for all compounds. Renal clearance was the only important excretory pathway for the compounds with a molecular weight < 200. Only the higher molecular weight compounds showed a profound bile/ plasma concentration ratio. Some of the high molecular weight compounds seem to be eliminated in bile from a compartment relatively slowly equilibrating with the plasma. For these compounds also an "uphill" excretion process into the gut lumen seems to be involved.

Correlations between lipophilicity and molecular weight on one hand and some pharmacokinetic parameters as clearance (Cl), elimination rate constant (k_{10}), volume of distribution (V) and terminal exponential slope (λ_z on the other hand are presented in Chapter III. The structure-pharmacokinetics relations were fitted by linear, parabolic or sigmoid curves. The relationship between total plasma clearance, hepatic, renal and intestinal clearance, and lipophilicity could be described most properly by the equation $Y = 1 / (aX^b + c)$ where Y stands for the logarithm of the relative pharmacokinetic parameters (Cl, k_{10}, V, λ_z)and X represents the logarithm of the relative values of some physicochemical parameters, such as the partition coefficient (P), the (HPLC) capacity factor k' and the molecular weight (MW). Hepatic and intestinal clearances in relation to the lipophilicity showed similar sigmoid correlation patterns, in contrast to the renal clearance, which by all means correlated poorly with lipophilicity.

It is concluded that lipophilicity is a better physicochemical parameter than molecular weight for the prediction of the pharmacokinetic behaviour of QAC's.

Calculation of the clearance (plasma clearance or total body clearance) can be performed on the basis of various concepts. Each of them is based on certain assumptions. For instance such calculations may be model-dependent or model-independent. Various methods for the calculation of the clearance were considered theoretically in Chapter IV. Using several equations the clearance values of

four closely related quaternary ammonium compounds were calculated from the experimental observations and the differences between the obtained values were discussed. Appropriate definition of the distribution volume of the peripheral compartment in an open two compartment model is crucial. If a drug is cleared from the peripheral compartment the apparent volume of this compartment is dependent on the excretion rate. Calculation of the clearance from the excretion rate and the plasma concentration ($Cl = \frac{dAe}{dt} / C_p$) appeared to be correct only if the drug is cleared from the central compartment. When elimination occurred from the peripheral compartment the $\frac{dAe}{dt} / C_p$ clearance values were highly variable with time, due to non-constant concentration ratios between V_1 and V_2, also in the terminal phase of the plasma disappearance curve. Discrepancy between clearance values based on rate constants and distribution volumes ($k_{10} \times V_1$ or $k_{20} \times V_2$) with those based on the calculation via $\frac{dAe}{dt} / C_p$ can be a diagnostic to establish elimination from a peripheral compartment.

Three quaternary ammonium compounds (QAC's) with different lipophilicity: triethylmethyl ammonium iodide (TEMA), tripropylmethyl ammonium iodide (TPMA), and tri-n-butylmethyl ammonium iodide (TBuMA) were chosen from this series of QAC's for more detailed hepatic transport studies (Chapter V).

They were given as a bolus injection of 10 μmole and 1 μmole in an isolated perfused liver. Two compounds showed saturation kinetics at D = 10 μmole, but not when 1 μmole of the agents was given. Biliary clearance of TEMA was equal to the bile flow, whereas for TPMA and TBuMA much higher values were found. Bile - to- liver ratios increased with a factor of 10, proportional to the lipophilicity of the compounds. Liver - to- plasma ratios of all three QAC's exceeded a value of 4, indicating that accumulation in the liver cannot be solely explained by passive equilibration according to the membrane potential. Transport into the bile appeared to be a concentrative process for TBuMA only. It was concluded that QAC's with a low lipophilicity such as TEMA, can be transported from plasma into bile by a passive process, which probably occurs paracellularly. An increased rate of uptake into the liver of such compounds does not result in an elevation of the biliary output. QAC's with an intermediate or a high lipophilicity are probably transported by carrier mediated processes both at the level of hepatocyte uptake

and bile canalicular transport.

The influence of choleresis was investigated using sodium taurocholate (Tc). The excretion rate of TEMA appeared to be unaffected, whereas the biliary output of TPMA and TBuMA was clearly elevated when the bile flow was increased. The latter effect was explained by an increase in the net flux across the canalicular membranes due to a decreased canalicular concentration. Also ion pair formation between taurocholate and the QAC's may play a role.

This aspect was further studied with the anorganic anion iodide (I^-) and an organic anion tetraphenylborate (TPB^-) (Chapter VI).

In the perfusion medium 25% of the amount of NaCl was replaced by NaI, whereas in two other series of experiments TPB^- was added to the medium in two concentrations (2μM and 200 μM). NaI did not affect the biliary output of the three QAC's although an increased net rate of hepatic uptake was found for all compounds. Liver - to - plasma concentration ratios were increased, while the ratios between bile and plasma were not affected.

TPB^- in catalytic amounts added to the medium (2μM) somewhat decreased the biliary output of TEMA and TBuMA, whereas the kinetic profile of TPMA was basically unchanged. A large amount of TPB^- (200 μM), added to the perfusion medium, dramatically changed the kinetic profile of the three QAC's. Ion pair formation between the QAC's 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 however was greatly reduced in spite of a normal bile flow. The rate of liver uptake of the QAC's was enhanced in the presence of the anion, which was due to an increase in plasma to liver transport (k_{12}) and a reduced liver to plasma transport (k_{21}). This increased net uptake into the hepatocytes however did not result in an increased biliary output. In fact the effects of TPB^- on the biliary output were strongly negative depending on its concentration.
The remarkable decrease in biliary transport of the QAC's caused by TPB^- in high concentration can in principle be explained by reduction of the effective intracellular concentration for the canalicular transport, due to ion pair formation and/or an increased intracellular binding. Alternatively this effect might be the consequence of an increased

bile to liver transport (increased biliary reabsorption) of the uncharged ion pair.

In Chapter VII the preliminary results are given of transport experiments with TBuMA using an isolated perfused intestinal loop preparation in situ. The rapid uptake into the intestinal wall and relatively slow secretion into the gut lumen resemble the kinetic behaviour in the liver, in accordance with the clearance - lipophilicity correlations described in Chapter III.

The present data on the relation between the chemical features and pharmacokinetic parameters of organic cations may contribute to a better understanding of the transport mechanisms involved in the elimination and distribution of drugs. This may also improve anticipation of posssible interactions between basic drugs at the level of membrane transport in the excretory organs.