abscissa. At low sulfinpyrazone levels the slope of the curve is −1; the decrease is equal to the molar displacer concentration, indicating that sulfinpyrazone is tightly bound and that each molecule of it occupies the same binding area as one molecule of warfarin. Sulfinpyrazone thus shows a maximal warfarin displacing effect.

The significance of this finding for the possible displacing effect in the clinical setting can be evaluated as follows. The ratio of free/bound warfarin is inversely proportional to the albumin reserve (Brodersen et al 1982). The maximal plasma level of sulfinpyrazone obtained at an oral dosage of 800 mg daily to adult females was 50 μM, in a study by Rosenkranz et al (1983). From Fig. 1 we read that at this sulfinpyrazone level the albumin reserve is decreased from 300 to 260 μM. The free warfarin fraction is therefore increased by a factor 300/260 = 1·15. This result depends upon the albumin concentration used in the test; a higher albumin would give a lower increase of free warfarin fraction. We have chosen a low concentration, 300 μM, as in hypoalbuminaemic patient in order to be on the safe side. In a healthy individual the increase of free warfarin would rather be by a factor about 1·05 to 1·1.

Sulfinpyrazone, in spite of its maximal warfarin displacing potential, should thus produce displacement to a moderate or insignificant degree at clinically relevant plasma levels. This is in agreement with the findings of O'Reilly & Goulart (1981). At lower sulfinpyrazone dosage, 400 mg daily, these authors could not demonstrate an increase of free warfarin in healthy volunteers.

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o- and *m*-Iodohippurate binding to plasma proteins as a model drug transport mechanism

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The pharmacokinetics of two isomers, *o*- and *m*-iodohippurate, were determined in rabbits and rats and the effect of protein binding on their elimination is demonstrated. Both isomers are rapidly eliminated by transport systems in the kidney and their clearance by the kidney approaches the renal plasma flow regardless of protein binding, *m*-Iodohippurate is more highly bound to plasma proteins than *o*-iodohippurate and its rate of elimination is enhanced in comparison with *o*-iodohippurate. In the case of these two isomers, the binding to plasma proteins should be considered as a transport mechanism and not as a storage depot.

The assumption that drug-plasma protein binding can serve as a transport system is generally accepted and the effect on drug elimination calculated (Gillette 1973), but evidence of how the mechanism of drug-plasma protein binding affects the pharmacokinetics of a drug is scanty.

This communication is concerned with a comparison of pharmacokinetic parameters of *o*- and *m*-iodohippurate, two isomers with similar biodistribution (Lázníček et al 1984) but different binding to plasma proteins, in rats and rabbits. For analysis of the mechanism of elimination of both isomers, their clearances are compared with that of inulin, which is generally accepted as a standard for glomerular filtration rate measurement (Blaufox et al 1975). The effect of plasma protein binding on pharmacokinetic parameters of the isomers is discussed.

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Material and methods

Substances used. [^{125}I]*o*-Iodohippurate and [^{125}I]*m*-iodohippurate (Nuclear Research Centre, Řež near Prague) specific activity of 2 GBq g^{-1}, dissolved in 0.9% NaCl (saline), radiochemical purity over 97.5%.

[Methoxy-^{14}C]methoxyinulin (ÚVVVR Prague) specific activity 1.5 GBq g^{-1}, radiochemical purity over 98%.

Rate studies. Wistar strain male rats (170–220 g) were fasted for 18–24 h before the experiment, water was freely available. The rats were dosed intravenously

(into the exposed femoral vein under light ether anaesthesia) with 0.5 mg kg^{-1} of 'drug'. During the course of the experiment the rats were housed singly in cages. At selected times after dosing, they were anaesthetized with ether, a blood sample withdrawn from the exposed carotid artery and then they were exsanguinated. The kidneys were excised to determine the ^{125}I activity.

Rabbit studies. For pharmacokinetic studies, unanaesthetized male rabbits of the grey Chinchilla strain (2.7–3.5 kg) were used. Compounds were administered into the right marginal vein at a dose of 0.1 mg kg^{-1}. Heparinized blood samples were obtained by means of permanent catheterization of the left marginal vein at selected times. The total amount of blood taken never exceeded 4 ml/animal.

For renal clearance experiments, rabbits were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). After a mid-abdominal incision, polyethylene catheters were inserted into the ureters for urine collection. Iodohippurate (100 μg kg^{-1}) and inulin (200 μg kg^{-1}) were infused intravenously in 1% mannitol at (0.5 ml min^{-1} 5 μg min^{-1}) through a cannulated femoral vein. Blood samples were obtained from the cannulated carotid artery. Urine was collected during a 10 min period (started 30 min after the primary dose), and a blood sample was taken at the midpoint of the urine collection period. At the midpoint of the second urine collection period, blood samples were withdrawn from the left and right renal vein to determine the renal extraction ratio. Blood withdrawn at the same time from the carotid artery was used for the in-vitro determination of drug-plasma protein binding. Urine flow rate was increased by mannitol injection (5 ml of 10% mannitol i.v.) 15 min after the inulin or iodohippurate.

Renal clearance (CL_R) in ml min^{-1} was calculated as

$$CL_R = V.V_{ur}.C_p^{-1}$$

where V, and C_{ur} and C_p indicate urine flow rate in ml min^{-1}, and urine and plasma concentration of the 'drug' in mg ml^{-1}, respectively. Renal extraction ratio E_R was calculated as

$$E_R = (C_{pa} - C_{pv}).C_{pa}^{-1}$$

where C_{pa} is concentration of 'drug' in plasma from arterial blood and C_{pv} is concentration of 'drug' in plasma from the renal vein.

Protein binding in-vitro. The binding of *o*- and *m*-iodohippurate to rat and rabbit plasma was determined by equilibrium dialysis at 37°C. Plasma was obtained by centrifugation of heparinized blood. Plasma (0.5 ml) was dialysed against the same volume of saline buffered with 0.01 mol $litre^{-1}$ phosphate (pH 7.4) with a test substance at the initial concentration of 1 mg $litre^{-1}$ for the period necessary to establish the dialysing equilibrium (2 h).

Radiometric determinations. The activity of ^{125}I in biological samples was measured on the beta-gamma spectrometer NE 8312 (Nuclear Enterprises Ltd, Edinburgh) and compared with the activity of standard samples of ^{125}I-labelled substances.

The activity of ^{14}C in urine and plasma 0.1 ml was counted by liquid scintillation (NE 8312) directly in 10 ml of Bray scintillation cocktail (Spolana, Neratovice). Quench correction was performed by the external standard method.

Pharmacokinetic analysis. The pharmacokinetic parameters were calculated using a programmable Sharp PC-1211 calculator. The post-injection plasma concentration-time courses were analysed by linear regression analysis of iterative non-linear least squares regression analysis. In rats, the one compartment open model [Wagner 1975] and equation

$$C = C(O) \exp(-\lambda_2 t)$$

was used for concentration-time data following 'drug' administration, where C and C(O) are concentration of 'drug' at time t and zero, and λ_z is disposition rate constant. In rabbits, experimental data could be fitted to the function

$$C = C_1 \exp(-\lambda_1 t) + C_z \exp(-\lambda_2 t)$$

which is the representative of the linear two-compartment open pharmacokinetic model (Wagner 1975), where C_1 and C_z are the zero time, hybrid concentration constants, while λ_1 and λ_2 are the hybrid rate constants corresponding to the phases of distribution and elimination, respectively.

Results

In rats, the plasma activity of ^{125}I-time courses in the interval of 1–45 min after administration of [^{125}I]*o*-iodohippurate and [^{125}I]*m*-iodohippurate is approximately monoexponential (Fig. 1). Over longer time intervals, when the activity of ^{125}I in plasma is lower than 2% of the initial plasma activity, a deviation from the mono-exponential course occurs. However, the activity of ^{125}I in these longer time intervals may be formed to a great extent by radiochemical impurities and/or metabolites of the original substances (Boegl & Stockhausen 1978).

The activities of ^{125}I calculated from plasma concentrations of *o*-iodohippurate and *m*-iodohippurate in the interval up to 45 min after their administration formed the basis for the calculation of pharmacokinetic

Table 1. Pharmacokinetic parameters of *ortho*-iodohippurate and *m*-iodohippurate in rats.

Parameter	*o*-Iodo-hippurate	*m*-Iodo-hippurate
Total body clearance of drug from plasma CL (ml min^{-1} kg^{-1})	10.78	9.83
Volume of distribution V_c (ml kg^{-1})	152	113
Elimination half-life $t_{\frac{1}{2}}$ (min)	9.75	7.98
Free fraction of drug in plasma	0.64	0.11

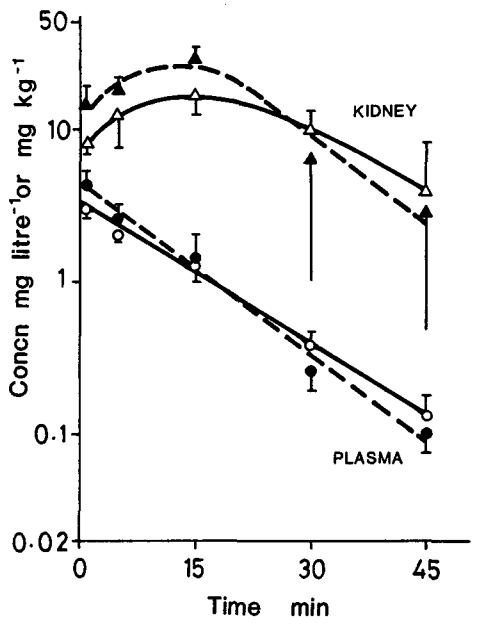

FIG. 1. Mean plasma (circles) and kidney (triangles) levels of *o*-iodohippurate (open symbols and solid lines) and *m*-iodohippufate (closed symbols and dotted lines) after intravenous administration of 0.5 mg kg^{-1} in rats. Each point represents mean ± s.d. of six animals.

parameters of the substances by means of linear regression analysis. Table 1 shows the calculated values of these parameters and the binding to plasma proteins.

Though both *o*-iodohippurate and *m*-iodohippurate at physiological pH are hydrophilic (partition ratio between n-octanol and 0.067 mol $litre^{-1}$ phosphate, pH 7.4, is for *o*-iodohippurate 0.028, for *m*-iodohippurate 0.098), the differences in their lipophility obviously result in the difference in their binding to plasma proteins. The higher plasma binding of *m*-iodohippurate is, then, evidently the cause of its higher initial plasma concentration resulting in a lower distribution volume (Vd) in comparison with *o*-iodohippurate. The lower distribution volume of *m*-iodohippurate at nearly identical value of the total body clearance of both substances results also in a lower half-life of elimination of *m*-iodohippurate, which is manifested in a more rapid decrease in its plasma concentration in comparison with *o*-iodohippurate (Fig. 1).

In rabbits, the sum of the pharmacokinetic parameters calculated from the time decrease in the plasma concentrations of the substances after their intravenous administration in the dose of 0.1 mg kg^{-1} is shown in Table 2. Pharmacokinetic parameters were calculated for every animal separately and the results represent the means and standard deviations of these individual values. The higher plasma binding of *m*-iodohippurate correlates with the finding of lower values of both the distribution volume of the central compartment and the distribution volume steady state in comparison with

Table 2. Pharmacokinetic parameters of *o*-iodohippurate and *m*-iodohippurate in rabbits (means ± s.d. from six determinations).

Parameter	*o*-Iodo-hippurate	*m*-Iodo-hippurate
Total body clearance of drug from plasma CL (ml min^{-1} kg^{-1})	18.0 ± 4.4	16.8 ± 4.4
Pharmacokinetic volume of central compartment V_c (ml kg^{-1})	160 ± 32	68 ± 17
Volume of distribution at steady state V_{ss} (ml kg^{-1})	259 ± 38	142 ± 37
Half-life associated with first exponent of biexponential equation $t_{\frac{1}{2}\lambda_1}$ (min)	4.1 ± 1.5	1.9 ± 0.5
Elimination half-life $t_{\frac{1}{2}\lambda_2}$ (min)	17.3 ± 2.4	13.5 ± 4.9
Free fraction of drug in plasma	0.60	0.05

o-iodohippurate. Of the minimum difference in the values of total plasma clearance of the isomers, the calculated values of the half-lives $t_{\frac{1}{2}\lambda_1}$ and $t_{\frac{1}{2}\lambda_z}$ are lower in *m*-iodohippurate. From the value of the glomerular filtration rate (defined as the total plasma clearance of inulin) and taking into account the plasma binding, it can be calculated that the proportion of glomerular filtration in the total elimination for *o*-iodohippurate represents approximately 12%, and for *m*-iodohippurate less than 2%. The higher plasma binding of *m*-iodohippurate thus results in its higher initial plasma concentration (smaller distribution volume of central compartment) and a more rapid decrease in these concentrations in comparison with *o*-iodohippurate (Fig. 2).

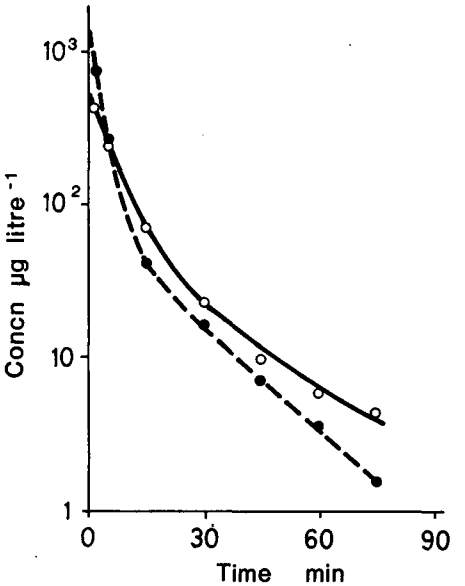

FIG. 2. Biexponential semilogarithmic plots of plasma concentrations of *o*-iodohippurate (○——○) and *m*-iodohippurate (●——●) in the same rabbit. Pharmacokinetics of *m*-iodohippurate was studied 5 days after *o*-iodohippurate.

Table 3. Renal clearance data of the left and right kidneys for *o*-iodohippurate and *m*-iodohippurate in the rabbit.

Drug	Time after loading (min)	Urine flow (ml min^{-1}) Left	Right	Renal clearance of inulin (ml min^{-1}) Left	Right	Renal clearance of iodohippurate (ml min^{-1}) Left	Right	Renal extraction ratio of iodohippurate Left	Right	Free fraction in plasm
Rabbit, male 2.8 kg										
o-Iodohippurate	30–40	0.20	0.13	6.2	7.5	27.0	30.7			
	40–50	0.07	0.04	5.4	5.5	27.9	27.5	0.88	0.86	0.53
	50–60	0.07	0.04	5.5	5.1	22.1	19.8			
Rabbit, male 2.9 kg										
m-Iodohippurate	30–40	0.08	0.07	7.8	6.5	26.0	20.0			
	40–50	0.05	0.06	5.6	7.1	18.6	21.4	0.87	0.88	0.06
	50–60	0.07	0.05	6.0	6.4	26.2	20.7			

The results obtained from renal clearance of the isomer in rabbits (Table 3) show that both regardless of their binding to plasma proteins, are extensively eliminated during their passage through the kidney, the renal extraction ratio being 0.86–0.88. Comparison with the value for the renal clearance of inulin with respect to plasma binding allows the proportion of glomerular filtration of the total amount excreted via the kidneys to be determined, for *o*-iodohippurate this is approximately 15%, and for *m*-iodohippurate, 2%. Both isomers are thus excreted predominently by tubular secretion. This fact is generally known for *o*-iodohippurate, as ^{131}I-labelled *o*-iodohippurate has proved to be a valuable compound for the measurement of effective renal plasma flow (Tubis et al 1960).

The determined value of renal clearance of *o*- and *m*-iodohippurate is slightly lower than the value of total plasma clearance calculated from the plasma-time courses; there are, however, differences both in the technique of the experiment (the effect of anaesthesia) and the manner of calculation.

Discussion

When the influence of plasma binding on the pharmacokinetic parameters of *o*- and *m*-iodohippurate is taken into account, it may be stated that binding to plasma proteins is not a limiting factor for the excretion of these isomers, which is mostly by tubular secretion (Table 3). Total plasma clearance of both isomers is substantially similar both in rats (Table 1) and rabbits (Table 2). But both in rats and rabbits there were differences between the two isomers in the plasma protein binding. Higher plasma binding of *m*-iodohippurate is manifested by a decrease in the magnitude of its distribution volume in comparison with *o*-iodohippurate in rats (Table 1) and rabbits (Table 2). As, at equilibrium, the concentration of free isomer in plasma and tissues is identical, an increase in the plasma binding results in a decrease in the isomer concentration in the tissues and in an increase in its concentration in the plasma. As the predominent amount of both isomers, regardless of plasma binding, is eliminated from the plasma during the passage through the kidney, the greater supply (higher plasma concentration) of that isomer more highly bound to plasma proteins (*m*-iodohippurate) results in its accelerated elimination from the plasma in comparison with the other isomers. *o*-iodohippurate.

Because of changes in the characteristics of the central compartment, corresponding changes occur in the character of the time course of the concentration of the isomers in the kidney. The higher plasma concentration of *m*-iodohippurate in the initial time intervals (higher supply) is reflected by its higher concentration in the kidney in the 15 min after administration, compared with *o*-iodohippurate. A more rapid decrease in plasma concentrations of *m*-iodohippurate with time also results in a more rapid decrease in its concentration in the kidney over longer times in contrast to *o*-iodohippurate (Fig. 1).

The results of this study of the effect of plasma binding of *o*-iodohippurate and *m*-iodohippurate on their pharmacokinetic parameters experimentally confirm a theoretically derived forecast (Gillette 1973) of the biological behaviour of the drugs, in which drug-plasma protein binding serves as the transport mechanism.

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