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METABOLISM AND BILIARY EXCRETION OF THE LIPOPHILIC DRUG MOLECULES, IMIPRAMINE AND DESMETHYLIMIPRAMINE IN THE RAT—I

EXPERIMENTS *IN VIVO* AND WITH ISOLATED PERFUSED LIVERS

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Abstract—After intraperitoneal administration of imipramine (IP) to rats with bile fistula, a large part of the material is excreted with the bile. The different types, their concentrations and their distribution of the IP-metabolites were measured as a function of time. Good agreement was obtained between intact, sham operated, and rats with fistulas as well as isolated perfused rat livers. Metabolic studies in the latter system were also performed with desmethylimipramine (DMI); in addition, the influence of bile salts, SKF 525-A and phenobarbital pretreatment on IP metabolism was studied. Besides large amounts of glucuronides the bile also contains unchanged IP and DMI. The bile/plasma concentration ratios of these two highly lipophilic compounds are in the order of magnitude of 50.

A WEALTH of observations has led to the generally accepted conclusion that foreign compounds, in order to be excreted in the bile, must have a molecular weight of more than about 300 and have a certain degree of polarity.[1] Anions of conjugates are particularly suitable for concentrative transfer into bile. In contrast, unchanged lipophilic drugs are assumed not to be excreted by this route, or to occur in bile in concentrations not exceeding the plasma levels. This behavior would be explained by a mere passive diffusion of the lipophilic molecules across the canalicular membranes of the hepatocytes, similarly to the behavior at other membranes.

Very few exceptions to this rule have been reported. Haddock *et al.*[2] found that within a series of iron chelates only the more lipophilic members were excreted into the bile of rabbits, the bile/plasma concentration ratio increasing up to 5. Meyer-Brunot and Keberle[3] reported that ferrioxamines are excreted into the bile of rats. The derivatives tested were neutral molecules not undergoing metabolic changes. Their rates of biliary excretion followed the lipophility of the compounds, the most lipophilic members reaching bile/plasma ratios of 100. An active transport was assumed by the authors.

Bickel and Weder[4] found that after parenteral administration of imipramine (IP) to rats more than half the dose can be localized in intestinal contents. The greater part consisted of glucuronides and other polar metabolites, although considerable amounts of IP and desmethylimipramine (DMI) were also present. This indicated a biliary excretion of lipophilic molecules.

The present study was undertaken to demonstrate this biliary excretion of the

lipophilic drug molecules conclusively and to study the kinetics of metabolism and biliary excretion. As an extension of the experiments of Bickel and Weder[4] the biliary excretion of unchanged IP and its metabolites was studied in rats with bile fistula and sham-operated controls. For a more detailed study the technique of the isolated perfused rat liver was used. In addition, the perfusion studies yielded useful data on the metabolism of IP under various conditions.

MATERIALS AND METHODS

Animals and drugs

Male Wistar rats of 210–300 g body weight were used. The hydrochlorides of imipramine (IP), desmethylimipramine (desipramine, DMI), desdimethylimipramine (DDMI), iminodibenzyl (IDB), 2-hydroxy-imipramine (2-OH-IP) and the fumarate of 2-hydroxy-desipramine (2-OH-DMI) were generously donated by Geigy Ltd., Basel, and imipramine-*N*-oxide by Dumex Ltd., Copenhagen. Other chemicals used are mentioned below.

Analytical methods

Extraction of tissues and biological fluids has been described in a previous paper.[4] The determination of all individual IP-metabolites in biological samples has been carried out by thin layer chromatography before and after hydrolysis of the conjugated fraction (glucuronides, -GA) by Glusulase Boehringer.[4] In some cases experiments or method testing were carried out by using 10-^{14}C-imipramine acquired from the Radiochemical Centre, Amersham, England. Tissues were then measured after combustion[5] and extracts directly by liquid scintillation counting using external standards for each type of experiment.

Rats with bile fistulas

Rats were anesthetized with 10–15 mg nembutal. For the canulation of the common bile duct a teflon canula was used since this material does not absorb imipramine (see below). A femoral vein was canulated for injections. After the operation the animals were placed in restriction cages. Sham-operated animals were subjected to laparotomy without bile duct canulation. Five min after completion of surgery the experiments were started by injecting drug intraperitoneally. In some experiments the animals were given 0·5 ml/hr bile from untreated rats with fistula or 5 mg/hr of a mixture of bile salts ("sodium taurocholate", Nutritional Biochemicals Corp., Cleveland, O.), both as intermittent i.v. injections every 15 min. The bile salt mixture contains cholate, deoxycholate, taurocholate, taurodeoxycholate, and taurochenodeoxycholate, 20 per cent each.[6] At the end of the experiments the animals were killed and the drug metabolites determined in bile, liver, plasma and small intestinal contents.

Liver perfusion

The procedures and apparatus described by Miller[7] were adopted with certain modifications. Preliminary experiments with ^{14}C-IP and a previously used apparatus[8] showed that due to extreme uptake by soft plastic materials[9] a total recovery of only a few percent resulted. In the modified perfusion apparatus depicted in Fig. 1 only glass or teflon parts and a nylon filter have therefore been allowed to be in contact with the perfusion medium. ^{14}C-recoveries of about 90 per cent were then obtained

with IP. The livers were perfused at 37° with 200 ml perfusion medium at a perfusion rate of 1·5 ml/min/g liver and and a portal pressure of 9 to 12 cm H_2O. A constant pH of 7·40–7·45 was achieved by oxygenation with a mixture of 97% O_2 and 3% CO_2 at a flow rate of 180 ml/min. The 200 ml of perfusion medium consisted of Krebs–Ringer-bicarbonate-solution containing washed bovine red cells (16 g hemoglobin), bovine

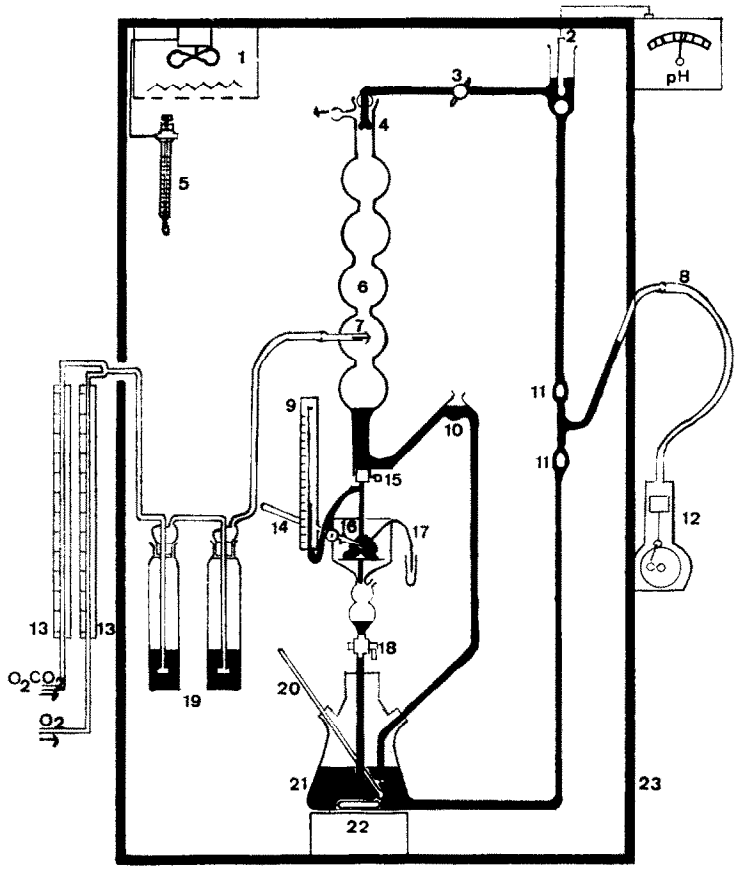

FIG. 1. Liver perfusion apparatus. 1: Heating, 2: electrode for pH-meter, 3: teflon tap, 4: perfusate distributor, 5: thermostat, 6: oxygenator, 7: gas inlet, 8: air cushion, 9: manometer, 10: overflow, 11: glass valves, 12: piston pump, 13: rotameters, 14: bile canula fixation device, 15: teflon tap with nylon filter, 16: liver chamber with liver, 17: bile collection, 18: teflon tap for flow rate measurement, 19: gas humidifiers, 20: thermometer, 21: perfusate reservoir, 22: magnetic stirrer, 23: box.

albumin (4·0 g), glucose (0·2 g) and aureomycine (16 mg). The drugs were added after 1 hr of perfusion and the experiments were carried out for an additional period of 3 hr. IP was added in concentrations of 5×10^{-5} M. Higher concentrations lead to prohibitive increase of hepatic perfusion resistance. The lactate/pyruvate coefficients determined[10] in the course of perfusion experiments varied between 7 and 12 (normal value — 10).

RESULTS

Rats with bile fistulas

Fistula rats were given 50 mg/kg IP i.p. Unchanged drug as well as metabolites were determined in the total collected bile, liver and plasma after 0·5, 1, 2 and 5 hr. The concentration values are listed in Table 1. The bile/plasma concentration ratios (Table 2) are based on the mean plasma concentrations calculated from the area under the plasma curves.

TABLE 1. CONCENTRATIONS OF IMIPRAMINE AND ITS METABOLITES (μg/g) IN RATS WITH BILE FISTULAS AFTER 50 mg/kg IMIPRAMINE i.p.

Compartments and metabolites	0·5 hr	1 hr	2 hr	5 hr
Plasma:				
IP	0·2	0·6	0·6	0·4
DMI	1	0·7	0·7	0·5
IPNO	0·2	0	0	0
2-OH-IP-GA	0	0·3	0	0
2-OH-DMI-GA	0	0·6	0·8	0·6
Liver				
IP	36	26	14	7
DMI	83	54	38	28
DDMI	5	4	3	0
2-OH-IP	2	10	7	0
2-OH-DMI	1	3	3	1
Bile:				
IP	11	26	27	22
DMI	8	18	10	48
DDMI	0	0	3	2
IDB	3	3	6	5
IPNO	0	7	2	2
2-OH-IP	11	9	11	17
2-OH-DMI	6	11	8	8
R-0·25	0	0	0	3
2-OH-IP-GA	182	197	140	128
2-OH-DMI-GA	82	142	187	108
R-0·25-GA	0	0	2	2

Only detected metabolites are listed. GA = glucuronic acid, R—0·25 = presumably 2-OH-DDMI.[4] Means of two rats per time point.

TABLE 2. BILE/PLASMA CONCENTRATION RATIOS IN RATS WITH BILE FISTULAS AFTER 50 mg/kg IMIPRAMINE i.p.

	0–0·5 hr	0–1 hr	0–2 hr	0–5 hr
Imipramine	129	87	60	46
Glucuronides	—	1210	587	404
Total drug	433	310	226	197

The bile flow of both normal and IP-treated rats averaged 0·5 ml/hr. Infusion of bile salts increase both choleresis and bile salt excretion by a factor of 1·5, but does not increase the amount of drug or metabolites excreted.

Figure 2 shows the amounts of total drug in bile and/or small intestinal contents of canulated vs. sham-operated rats. Whereas the metabolite pattern in small intestinal contents of sham-operated rats practically coincides with the one in fistula bile (Table 1),

the material found in the intestinal contents of rats with fistulas consists almost exclusively of IP and DMI.

The drug excreted in the bile is composed of a mean of 80% glucuronides and 5% unchanged imipramine. During the first hour 2760 μg of total drug are excreted with the bile. Based on an average molecular weight of 400 a transfer of about 0·7 μmoles/hr per 1 g liver is obtained. During the initial phase the transfer is likely to be twice as high.

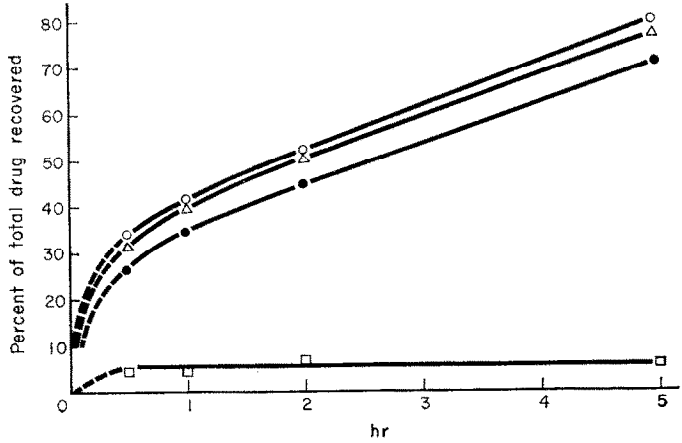

FIG. 2. Percentage of total drug recovered in liver and bile or small intestinal content (S.I.C.) respectively of sham operated and bile fistula rats given 50 mg/kg IP i.p. Fistula rats: □ S.I.C., ● bile, △ S.I.C. + bile. Sham operated rats: ○ S.I.C. Means of two experiments per type and time point.

Liver perfusion

Two rat livers were perfused with 200 ml perfusion medium to which 3·16 mg ^{14}C-IP (5×10^{-5} M) were added. Four ml samples of the medium were taken at various times up to 150 min and the radioactivity was counted in the total perfusate. The radioactivity of liver and total bile was counted after 150 min. Since the ^{14}C-atom in the 10-position is present in all metabolites and is not expired as $^{14}CO_2$,[11] the radioactivity corresponds to total drug. The mean recovery amounted to 91 per cent. Figure 3 shows the time curves for perfusion medium, bile and liver. The intermediate bile values were taken from experiments with unlabeled IP and slightly corrected to fit the terminal value obtained with ^{14}C-IP. The course of the liver curve represents the difference between 100 per cent and the sum of perfusate + bile values at each time point.

In perfusion experiments with unlabeled IP the unchanged drug as well as the individual metabolites were determined in the three compartments (Table 3). Glucuronides were not assayed in liver tissue since they have never been detected in a large number of previous experiments. No metabolites were formed in IP or DMI perfusions without a liver in the system. The bile/plasma concentration ratios (Table 4) are based on the mean plasma concentrations calculated from the areas under the plasma curves. A steady amount of 327 μg/hr of total drug is excreted with the bile, corresponding to 0·11 μmoles/hr/g liver when an average molecular weight of 400 is assumed. The drug

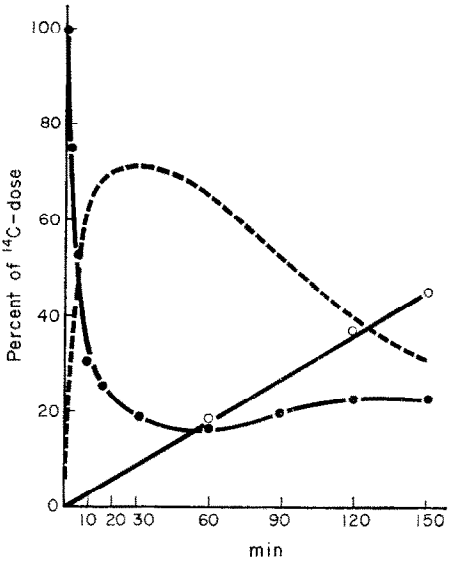

FIG. 3. Compartmental distribution of total drug in liver perfusion system after adding ^{14}C-IP. ● perfusate, ○ bile. Dotted curve: liver. Means of two experiments, corrected for 100 per cent recovery.

TABLE 3. RAT LIVER PERFUSIONS WITH IMIPRAMINE 5×10^{-5} M

Time (hr)	IP	DMI	DDMI	IDB	IPNO	2-OH-IP	2-OH-DMI	2-OH-IP-GA	2-OH-DMI-GA	R-0·25-GA
					(μg/g)					
Plasma*										
1	0·3	0·3	0	< 0·1	1	0·1	< 0·1	2	1	0
2	0·2	0·4	0	0·1	0·4	0·4	< 0·1	0·7	1	0
3	0·1	0·3	0	< 0·1	< 0·1	0·2	< 0·1	0·6	0·4	0
Liver										
3	22	58	12	0	0	2	2	—	—	—
Bile										
0–1	9	6	0·4	5	12	3	3	303	141	11
1–2	8	8	1	9	7	4	4	280	200	10
2–3	5	12	1	4	8	8	6	171	126	7

Mean values of five experiments. See also Table 1.
* Plasma of perfusion medium.

TABLE 4. BILE/PLASMA* CONCENTRATION RATIOS IN RAT LIVER PERFUSIONS WITH IMIPRAMINE 5×10^{-5} M

Substances	0–1 hr	1–2 hr	2–3 hr
Imipramine	5	32	33
Glucuronides	268	182	234
2-OH-metabolites	120	32	47
Total drug	76	133	145

* Plasma of perfusion medium.

TABLE 5. RAT LIVER PERFUSIONS WITH IMIPRAMINE (3·16 mg, $5 \cdot 10^{-5}$ M) UNDER VARIOUS CONDITIONS

	Control (5)				Bile salt infusion* (3)				Phenobarb. pretreatment† (5)				SKF 525-A† (4)			
	P	L	B	tot.	P	L	B	tot.	P	L	B	tot.	P	L	B	tot.
IP	1·1	10·7	0·5	12·3	1·2	13·4	0·8	15·4	1·0	9·8	0·6	11·4	3·8	18·5	1·4	23·7
DMI	3·4	29·3	0·5	33·2	3·8	25·8	0·8	30·4	2·5	28·8	0·4	31·7	0·8	38·0	0·1	38·9
IPNO	0 2	0	0·5	0·7	0·2	3·6	0·4	4·2	0	2·8	1·0	3·8	16·4	0	3·6	20·0
2-OH-‖	3·6	1·8	0·7	6·1	1·4	0·6	0·6	2·6	1·0	1·5	0·8	3·3	2·9	0	0·9	3·8
GAH-‖	11·3	—	30·0	41·3	14·0	—	28·2	42·2	17·1	—	25·6	42·7	1·4	—	7·7	9·1
Total	19 6	41·8	32·2	93·6	20·6	43·4	30·8	94·8	21·6	42·9	28·4	92·9	25·3	56·5	13·7	95·5

Distribution of metabolites % of dose after 3 hr.
* See Methods fistula rats, † five times daily 60 mg/kg i.p., 24-hr interval before experiment, ‡ 8·2 mg (10^{-4} M) 30 min before IP.
§ P = perfusate, L = liver, B = bile.
‖ 2-OH- = sum of hydroxylated metabolites, GA- = sum of glucuronides.
In brackets number of experiments.

contained in bile is composed of a mean of 92 per cent glucuronides and 2 per cent unchanged IP.

Table 5 is a synopsis of the results of IP perfusions under various conditions. The results are presented as distribution balances for the major metabolites in the three compartments after 3 hr of perfusion; they are calculated from the determined concentration values and corrected for a 100 per cent recovery. The difference of 100 per cent minus the sum total represents the minor metabolites, DDMI and IDB. The experiments with IP and normal livers mentioned above served as control. The normal bile flow rate of 0·4 ml/hr (with and without IP) is increased by a factor of 1·5 by the infusion of bile salts; however, the amount of drug excreted is not increased and the concentration therefore decreased. In addition Table 5 summarizes the results of IP perfusions with livers of phenobarbital-pretreated rats as well as with normal livers after coadministration of SKF 525-A to the perfusion medium. The results of the phenobarbital experiments may be influenced by the fact that the bile flow did not surpass a value of 0·3 ml/hr.

TABLE 6. RAT LIVER PERFUSIONS WITH DESIPRAMINE (3·02 mg, 5×10^{-5} M)

	Perfusate	Liver	Bile	Total
DMI	6·8	34·0	2·4	43·2
IP	0	1·2	0	1·2
DDMI	1·9	12·0	0·2	14·1
2-OH-*	3·9	0·4	0·6	4·9
GA-*	11·7	—	23·1	34·8
Total	24·3	47·6	26·3	98·2†

Distribution of metabolites (% of dose after 3 hr).
Mean values of six experiments.
* 2-OH- = sum of hydroxylated metabolites.
GA- = sum of glucuronides.
† 1·8% IDB.

Table 6 summarizes the results of perfusion experiments with DMI carried out and represented in the same manner as in Table 5. The steady state bile/plasma concentration ratios are approximately 65 for DMI, 200 for glucuronides, and 145 for total drug.

DISCUSSION

Metabolism of imipramine and desmethylimipramine

Since all detectable IP-metabolites were determined in the experiments with bile fistula rats and with perfused livers, these experiments furnish new data on IP-metabolism.

In a previous paper on the metabolism and distribution of IP in the intact rat[4] we reported that more than half the dose was localized in the small intestinal contents during the first hours after i.p. administration. This was interpreted as a result of abundant biliary excretion. Indeed, in the experiments with bile fistula rats similar amounts in a similar time-course are determined in the bile. In addition, Fig. 2 shows that the total drug in bile + intestinal contents of fistula rats practically coincides with the one found in intestinal contents of sham-operated animals. These values then equal

the sum of the values found in bile and in intestinal contents of fistula rats. Thus the major part of IP-like material found after parenteral administration in small intestinal contents of intact or sham-operated rats is due to biliary excretion and a small part seems to reach the lumen by extrabiliary secretion. Characteristically, the material found in the intestinal contents of fistula rats is composed of IP and DMI only. These most lipophilic molecular species are known for their ease of diffusion.[12] In the plasma of rats with bile fistulas (Table 1) and intact rats[4] the same metabolites in the same distribution pattern and time-course are found. The DMI plasma curves are virtually identical. Comparable tissue concentrations of the same metabolites are also observed in liver, except for small concentrations of 2-hydroxy-metabolites, present in the liver of animals with fistulas only. The bile of rats with fistulas contains the same molecular species in comparable amounts and time-course as the intestinal contents of sham-operated or intact rats. These results thus demonstrate that anesthesia, surgery and tapping of the bile does not significantly influence IP-metabolism and distribution under the experimental conditions chosen.

A good agreement also exists between the results obtained with rats with bile fistulas and with perfused rat livers. In plasma, liver, and bile of both systems (Tables 1 and 3) the same IP-metabolites are present in comparable concentrations and time-courses. The bile flow in the perfused liver was almost as high as in the fistula rat so that the amounts of metabolites excreted in the bile were again comparable in the two systems.

The closed system of the isolated perfused liver is suitable for the determination of total amounts of metabolites and thus metabolic rates. After 3 hr of rat liver perfusion with IP 88 per cent of the drug is metabolized. The same figure has been obtained for incubation with rat liver microsomes *in vitro*.[13] A comparison of the metabolite patterns shows that in the perfusion system there is less demethylation and more hydroxylation, whereby the major part of the hydroxylated metabolites are conjugated (Table 5). The same table shows that the use of livers from phenobarbital pretreated rats for the perfusion leads to virtually identical values, except for a certain increase in IPNO formation. The inhibitor of microsomal oxidative metabolism, SKF 525-A, decreases total IP-metabolism and inhibits hydroxylation by 73 per cent; *N*-oxide formation, which is not inhibited *in vitro*,[14] is strongly increased in the perfused liver. This is probably due to a metabolic shift from hydroxylation to *N*-oxidation. Strangely, SKF 525-A does not inhibit demethylation in the perfused liver, whereas *in vivo* as well as added to microsomes *in vitro* it inhibits this pathway.[15]

The metabolite and antidepressant drug, desmethylimipramine, is metabolized to only 57 per cent by 3 hr liver perfusion (Table 6). This is in agreement with the well known fact of DMI accumulation after IP administration in rats and humans. Forty per cent of the drug is hydroxylated—and most of it further conjugated—and 14 per cent is demethylated to DDMI. Finally, a small amount of IP has been detected. This *N*-methylation reaction of DMI, not observed *in vitro* with rat liver preparations,[13] has been found to occur in rabbit liver and lung by Dingell and Sanders.[16]

Liver perfusions with ^{14}C-IP (Fig. 3) show a rapid decline of radioactive material in the perfusion medium and a corresponding uptake by the liver. This reflects the situation *in vivo* but in a less extreme manner. *In vivo*, due to the presence of more tissues for uptake and of kidneys for elimination of plasma metabolites, the plasma level declines more rapidly and shows no increase in later phases.[4]

In conclusion, the comparison of metabolic and pharmacokinetic data of IP in intact rats, sham-operated and rats with bile fistulas, and in the isolated perfused rat liver shows a remarkably good agreement and proves the value of the latter system as a tool.

Biliary excretion

Administration of IP results in a near-linear excretion of about a third of the dose during the first 3 hr in the bile of both rats with fistulas and perfused livers (Figs. 2 and 3). As shown in Tables 1 and 3 glucuronides are present in very high concentrations in the bile (100–300 μg/ml). On the other hand free hydroxylated metabolites and IPNO occur in a concentration range of 2–15 μg/ml only. Both are minor terminal metabolites: IPNO may be an important short lived intermediate, and the hydroxylated metabolites are rapidly conjugated. Finally, the lipophilic molecular species IP and DMI are present in the bile in a concentration range of 5–45 μg/ml. The amount of unchanged drug excreted in the bile is only about 2 per cent of the dose in the perfused liver and 5 per cent in the fistula rat. In the case of IP this does not adequately reflect excretion capacity since the circulating amount of this compound is rapidly decreasing due to a high rate of metabolic transformation. The results summarized in Table 5 disclose the distribution of the major metabolites in the three compartments of the liver perfusion system. The lipophilic molecules, IP and DMI, are bound to the liver and therefore display plasma/liver concentration ratios of about 1:100 (Table 3). A similar ratio is observed in rats with fistulas (Table 1) and intact rats.[4] By this criterion the free hydroxylated metabolites are not bound in the liver cell but excreted both into bile and plasma. The glucuronides, which are present in high amounts in bile and plasma, cannot even be detected in the liver. Thus their excretion upon formation must be extremely rapid and efficient.

Substances excreted in the bile can be classified according to their bile/plasma concentration ratio c_b/c_p. Compounds showing a value of > 1 are called class B-compounds according to Brauer[17] or cholephils according to Hargreaves[18] and are supposed to be actively transported from the hepatocyte into the bile. The c_b/c_p values shown in Tables 2 and 4 for rats with bile fistulas and liver perfusions respectively are comparable for one molecular species and tend to stabilize with time. The glucuronides give ratios of several hundred or more. This confirms the data obtained with many other conjugates and reflects the active concentration mechanism for anions like glucuronidates. The terminal c_b/c_p values of unchanged imipramine are around 40. This demonstrates that there is a concentrative transport of this lipophilic molecule from plasma into bile. c_b/c_p values of around 30 and 25 respectively have been measured in the guinea pig (gall bladder bile) and in the dog (fistula bile).[19] With perfused rat livers under metabolic inhibition by SKF 525-A, c_b/c_p increased to 70 and in fistula rats during the first 30 min to over 100. Furthermore, if one considers that at least half of the plasma IP is albumin bound[20] and that, if back-diffusion in the biliary tree occurs the lipophilic IP is the most likely candidate, then the actual ratios between IP concentration in canalicular bile and free plasma concentration may even reach values in the order of magnitude of glucuronides. The c_b/c_p values determined for DMI, which is almost as lipophilic as IP,[12] are in the same order of magnitude as those for IP.

In fistula rats and liver perfusion experiments infusion of bile salts resulted in a

1·5-fold increase in both bile flow and bile salt excretion. However, the absolute amount of IP and DMI excreted was not influenced by the increase in bile volume and amount of bile salts. The amount of glucuronides was even decreased by 5 to 10 per cent, which may be due to a competitive inhibition by the bile salt anions.[21] This situation may indicate that the concentrative transport of glucuronide anions and lipophilic IP derivates are due to different mechanisms. The possible mechanism of the concentrative transport of the latter substances will be discussed in part II of this paper.[22]

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