Biochemical Pharmacology, Vol. 46, No. 6, pp. 983–991, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

METABOLISM OF ANTIPYRINE *IN VIVO* IN TWO RAT MODELS OF LIVER CIRRHOSIS

ITS RELATIONSHIP TO INTRINSIC CLEARANCE *IN VITRO* AND MICROSOMAL MEMBRANE LIPID COMPOSITION

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(*Received* 28 *September* 1992; *accepted* 8 *June* 1993)

Abstract—Antipyrine metabolism depends on at least three isoenzymes of cytochrome P450 forming the main metabolites 3-OH-, 4-OH- and norantipyrine. We investigated to what extent antipyrine clearance and metabolite formation are impaired in two models of liver cirrhosis in the rat, namely micronodular cirrhosis induced by chronic exposure to phenobarbital/CCl_4 and biliary cirrhosis induced by bile duct ligation. Salivary antipyrine clearance was decreased to a similar extent in cirrhosis induced by CCl_4 and bile duct ligation (−35%). Clearance for production of 3-OH-antipyrine was decreased in both models, while 4-hydroxylation was maintained. Metabolic clearance of both 3-OH-antipyrine and 4-OH-antipyrine *in vivo* correlated with their clearance *in vitro* ($r = 0.658$ and $r = 0.583$) but not with that of norantipyrine. The microsomal cholesterol content was increased by 16% and 90% in CCl_4 and bile duct-ligated cirrhotic rats ($P < 0.001$), respectively. Membrane fluidity, expressed as the ratio of phospholipids to cholesterol, correlated with the *in vivo* clearance for production of norantipyrine ($r = 0.841$) but not of 3-OH- or 4-OH-antipyrine, while clearance *in vitro* was not related to altered lipid composition. Our results demonstrate that the cytochrome P450 isoenzymes responsible for the different pathways of antipyrine metabolism are affected to different extents by cirrhosis. Alterations in intrinsic clearance explain only part of the loss of hepatocellular function. Altered lipid composition contributes to this loss of function but other factors, among them loss of hepatocytes and changes in microcirculation, could be more important determinants of the decrease in xenobiotic metabolism in cirrhosis.

Clearance of endo- and xenobiotics is one of the main functions of the liver; it is determined by hepatic perfusion and intrinsic clearance, a measure of the metabolic capacity of the liver [1]. The clearance of low extraction compounds such as antipyrine is mainly determined by the metabolic capacity of the mixed function oxidase system [1]. Antipyrine was one of the first model drugs used [2] and has remained a popular tool for metabolic studies to probe hepatic metabolic capacity [3]. Antipyrine is metabolized by different cytochrome P450 isoenzymes forming three major metabolites [4, 5]. Formation of 4-OH-antipyrine has been ascribed e.g. to cytochrome P450 IA2 but other isozymes involved in antipyrine metabolism have yet to be identified and might differ between rat and man [4]. Breimer [5] proposed that changes in the urinary excretion of the three main metabolites could increase the diagnostic value of the antipyrine test [5]; indeed, Teunissen *et al.* [6] described not only a decrease in antipyrine plasma clearance but also a markedly changed antipyrine metabolite pattern in subjects with alcoholic liver disease. These changes were difficult to evaluate since the groups varied with respect to age, smoking habits and concomitant drug intake, factors known to influence antipyrine metabolite formation [4, 7].

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Information on the activity of the different metabolic pathways *in vitro* and their correlation with metabolism *in vivo* is scarce, however [8], and only one study addresses this subject in cirrhosis [9]. The clearance of many drugs metabolized by cytochrome P450, including antipyrine, is reduced in cirrhosis [6, 9]. The activity of integral membrane enzymes such as cytochrome P450 often depends on the membrane lipid composition [10, 13]. Indeed, purified cytochrome P450 3A from rat and man requires specific lipids for functional reconstitution *in vitro* [13]; other isozymes appear less susceptible to membrane lipids. Lipid composition of microsomes of cirrhotic rats is altered [14–17] but it remains unclear whether these changes affect the function of cytochrome P450 *in vivo*.

Therefore, we investigated the metabolism of antipyrine in two models of cirrhosis in the rat, namely micronodular cirrhosis induced by chronic exposure to phenobarbital and CCl_4 [18], and biliary cirrhosis induced by ligation of the common bile duct [19].

MATERIALS AND METHODS

Chemicals. Antipyrine was from Fluka (Buchs, Switzerland), 4-hydroxy- and norantipyrine were from Aldrich (Milwaukee, WI, U.S.A.). 3-Hydroxymethyl-antipyrine was kindly supplied by Prof. Dr D. D. Breimer (Leiden, The Netherlands). Limpet acetone powder type I was from the Sigma Chemical

Co. (St Louis, MO, U.S.A.). Glucose-6-phosphate dehydrogenase grade II and NADP were from Boehringer (Mannheim, F.R.G.). [^{14}C]Dimethylaminoantipyrine was from Amersham International (U.K., sp. act. 120 mCi/mmol). Phospholipid standards were from Supelco (Bellefont, U.S.A.). HPLC grade methanol, dichloromethane and acetonitril were from Rathburn (Walkerburn, U.K.). All other chemicals were of analytical grade from different commercial sources.

Induction of cirrhosis. Male Sprague–Dawley rats (Deutsche Versuchstierfarm Hartmutt-Voss, Tuttlingen, F.R.G.) were maintained on standard laboratory chow (Kliba-Futter, Basel, Switzerland) and tap water under a 12 hr light–dark cycle at 20–22° and 50–60% humidity. Micronodular cirrhosis was induced in rats weighing 150–180 g by chronic exposure to phenobarbital and carbon tetrachloride for 16 weeks according to McLean *et al.* [18] as modified in our laboratory [20]. Treatment was withheld 2 weeks before the study, a time sufficient for the phenobarbital effects to disappear [20, 21]. Untreated littermates served as controls. Secondary biliary cirrhosis was produced by bile duct ligation [19] in animals weighing 300–400 g at the time of surgery as described previously [22]. Sham-operated animals served as controls. Animals were characterized with the aminopyrine breath test (ABT) as described previously [23, 24]; its results will be reported as the area under the curve of breath radioactivity from 0–2 hr (ABT_{AUC}). Serum bile acid levels were estimated by a commercially available radioimmunoassay (Becton-Dickinson, Orangeburg, SC, U.S.A.).

Although each cirrhotic group had its appropriate control, no difference in body, liver or spleen weight or ABT_{AUC} was found between the two control groups; therefore, the control groups were pooled and treated as one group.

Experimental design. The same experimental design as published previously [24] was used. Briefly, on day 1 antipyrine 40 mg/kg was given i.p. (40 mg/mL in 0.9% saline); the animals were placed in metabolic cages and urine was collected while allowing free access to food and water. Urine was collected for two 24 hr periods in collecting vials containing sodium pyrosulfite, acetate buffer pH 4.5 and sodium azide. On day 3 antipyrine was given again (40 mg/kg, i.p.) and saliva samples were obtained at 3, 5, 7 hr, each time 5 min after pilocarpine nitrate stimulation (2–4 mg/kg, i.p. [25].

Microsomal assays. On day 4 the animals were anesthetized with pentobarbital (50 mg/kg, i.p.). A blood sample was obtained from the vena cava. The portal vein was perfused with 20 mL ice-cold saline and the animal killed by exsanguination. Liver and spleen were removed, weighed and stored on ice. A 10 g portion of the liver was placed in ice-cold 0.1 M phosphate buffer (pH 7.4) and homogenized with 10 strokes of a loose-fitting glass homogenizer. The 100,000 *g* microsomal fraction was prepared by differential centrifugation and assayed within 4 hr for cytochrome P450 content [26], NADPH-dependent cytochrome *c* reductase activity [27], protein content [28] and antipyrine metabolite formation.

For the assay of 3-OH-, nor- and 4-OH-antipyrine formation the mixture contained about 1 mg/mL microsomal protein, antipyrine and a NADPH generating system consisting of 5 mM glucose-6-phosphate dehydrogenase in a total volume of 1 mL (pH 7.4); 10 concentrations of antipyrine ranging from 0–16 mM were studied [24]. The NADPH generating system containing antipyrine and the microsomes were preincubated separately for 5 min. The microsomes were added to the system and the mixture was incubated in a shaking water bath for 10 min at 37°.

The reaction was stopped with 333 μL 15% trichloroacetic acid. Sodium pyrosulfite and 4-methylbenzoic acid were added as antioxidant and internal standard, respectively. The vials were stored on ice and analysis was started within 15 min.

Assay of antipyrine in saliva, urine and microsomes. The HPLC methods used have been published before [24]; in brief, butalbital was added as an internal standard to 40 μL saliva which was then extracted with 1 mL dichloromethane. The organic layer was evaporated in a Buechler vortex evaporator and the residue redissolved and injected on a 100 × 3 mm MOS Hypersil^{R} column (Shadon, Southern Instruments Inc., Astmoor, U.K.). The HPLC system consisted of a Waters M45 pumping device and a WISP 712 automatic sample injector; a Kratos Spectraflow 757 variable UV detector set at 244 nm and a Merck-Hitachi D-2000 integrator. A standard curve with antipyrine concentrations ranging from 0 to 100 μg/mL (7 points) was obtained for each assay.

For the analysis of urine the method developed by Teunissen [29] was modified: duplicate samples were hydrolysed with 10 mg limpet acetone powder at 37°; 100 mg sodium pyrosulfite was added as an antioxidant. After 3 hr incubation, 200 mg NaCl was added and the sample extracted with 5 mL chloroform/ethanol (9:1). The organic phase was evaporated under reduced pressure. The residue was redissolved in 100 μL methanol and 200 μL 0.02 M acetate buffer pH 4.5 containing 30 mg/mL sodium pyrosulfite; 15 μL were injected on a Macherey–Nagel phenyl-coated silica column (end-capped, 125 × 4 mm, 5 μm). Otherwise, equipment as described above was used. Each day a standard curve ranging from 12.5 to 100 μg/mL for 3-OH and 4-OH-antipyrine and from 5 to 40 μg/mL for norantipyrine was obtained.

To quantitate antipyrine metabolites in microsomes the 1000-fold excess of antipyrine was removed at pH 11 with 5 mL toluol dichloromethane (7:3) at 4°. The organic layer was discarded and acetate buffer pH 4.5 was added at room temperature. The sample was applied to preconditioned solid phase columns. The columns were quantified as published previously [24]; in brief, the columns were washed successively with acetate buffer pH 4.5, water (both containing sodium pyrosulfite) and hexane; then, the metabolites were extracted with methanol. After evaporation the residue was redissolved and 15 μL were injected into a Macherey–Nagel phenyl column (250 × 4 mm, 7 μm) heated to 35°. The recoveries

Table 1. Organ weight (g), aminopyrine breath test (ABT_{AUC}) and serum bile acid concentration (μmol/L) in control animals (CTR; N = 14), cirrhosis induced by CCl_4 (CCl_4; N = 10) and bile duct-ligated rats (BDL; N = 7)

	Body weight	Liver weight	Spleen weight	ABT_{AUC}	Serum bile acids
CTR	601 ± 60	19.8 ± 3.4	1.1 ± 0.3	32.4 ± 5.1	1 ± 1
CCl_4	543 ± 78	19.1 ± 4.8	2.5 ± 0.7	19.2 ± 7.3	22 ± 9
BDL	537 ± 43	26.6 ± 6.8	3.5 ± 1.9	23.7 ± 4.5	57 ± 21
Significance					
CTR vs CCl_4	NS*	NS	<0.001	<0.05	<0.001
CTR vs BDL	<0.05	<0.05	<0.001	<0.05	<0.001
CCl_4 vs BDL	NS	<0.05	NS	NS	<0.001

Means ± SD are given; the groups were compared by Peritz' F-test.
* Not significant by Peritz' F-test.

Table 2. Pharmacokinetics of antipyrine elimination in saliva of control animals (CTR), cirrhosis induced by CCl_4/phenobarbital (CCl_4) and bile duct-ligated rats (BDL)

	$T_{1/2}$ (min)	V_d (l/kg)	Cl (mL/min/kg)
CTR	178 ± 47	0.89 ± 0.11	3.70 ± 1.02
CCl_4	260 ± 92	0.79 ± 0.08	2.33 ± 0.78
BDL	269 ± 71	0.87 ± 0.09	2.42 ± 0.81
Significance			
CTR vs CCl_4	<0.01	<0.05	<0.001
CTR vs BDL	<0.01	NS	<0.01
CCl_4 vs BDL	NS*	NS	NS

Means ± SD are given; the groups were compared by Peritz' F-test.
* Not significant by Peritz' F-test.

of 3-OH-, nor- and 4-OH-antipyrine from spiked microsomes were 109, 98 and 83%, respectively. Standard curves ranging from 0 to 4 μg/mL 3-OH-antipyrine, 0 to 3 μg/mL norantipyrine and 0 to 6 μg/mL 4-OH-antipyrine were obtained every day.

Lipid analysis of microsomes. Microsomal lipids were extracted according to Bligh and Dyer [30] using coprostanol as an internal standard. Cholesterol was determined by GLC on an OV73 column as described and referenced [17]. Total phospholipids were quantitated according to Bartlett [31]. Individual phospholipids were separated by high performance TLC and quantified as described [17].

Calculations. Preliminary experiments taking saliva samples up to 10 hr had demonstrated that in cirrhotic rats the disappearance of antipyrine from saliva could also adequately be described by a one-compartment open model in agreement with other authors [32]. Half-life ($T_{1/2}$) was determined for each animal by least-squares regression analysis of the log-linear saliva concentration versus time curve. The volume of distribution (V_d) was determined by dividing the dose by the extrapolated antipyrine concentration at time = 0. The clearance (Cl) was calculated as:

$$Cl = \frac{\ln 2 . V_d}{T_{1/2}}. \quad (1)$$

The amount of metabolite excreted in urine was expressed as molar % of the dose. Partial clearance for production of the metabolites (Cl_m) was calculated as the product of the total clearance (Cl) and the fraction of the dose excreted as that metabolite (f_m) by the equation [33]:

$$Cl_m = Cl . f_m. \quad (2)$$

The apparent V_{max} and K_m of microsomal enzyme kinetics were calculated by non-linear fitting to the Michaelis–Menten equation [34]. Microsomal intrinsic clearance (Cl_{int}) was calculated as the ratio V_{max}/K_m and total hepatic cytochrome P450 content (P450):

$$Cl_{int} = \frac{V_{max}}{K_m} . P450. \quad (3)$$

Total hepatic cytochrome P450 content was corrected for the difference in protein content and liver size.

Statistical analysis. All results are expressed as means ± one standard deviation. Differences between the three groups were evaluated for statistical significance by Peritz' F-test [35]. Linear regression analysis was carried out by the method of least squares [36]. $P < 0.05$ was considered statistically significant.

Table 3. Urinary metabolic clearance for production (mL/min) of 3-OH- (3-OH), nor- (NORA) and 4-OH- (4-OH) antipyrine in control animals (CTR), cirrhosis induced by CCl_4 (CCl_4) and bile duct-ligated rats (BDL)

	3-OH	NORA	4-OH
CTR	0.53 ± 0.19	0.20 ± 0.08	0.31 ± 0.11
CCl_4	0.29 ± 0.15	0.07 ± 0.04	0.27 ± 0.09
BDL	0.31 ± 0.16	0.12 ± 0.08	0.24 ± 0.06
Significance			
CTR vs CCl_4	<0.01	<0.001	NS
CTR vs BDL	P < 0.05	NS	NS
CCl_4 vs BDL	NS*	NS	NS

Urinary metabolite excretion in % of dose is given in the text.

Means ± SD are given; the groups were compared by Peritz' F-test.

* Not significant by Peritz' F-test.

RESULTS

The characteristics of the three groups are reported in Table 1. Bile duct-ligated rats weighed 10% less but had 25% increased liver weight compared to control rats ($P < 0.05$). The body and liver weights of CCl_4-cirrhotic rats were not different from controls (Table 1). All treated animals had cirrhosis of the liver: ABT_{AUC} was reduced in both cirrhotic groups; all treated animals had portal hypertension as evidenced by dilated splanchnic veins and splenomegaly (Table 1). Serum bile acids were higher in both cirrhotic groups ($P < 0.001$), the bile duct-ligated cirrhotics having higher serum levels than the CCl_4-cirrhotic animals (Table 1, $P < 0.001$).

The results of antipyrine elimination from saliva are shown in Table 2. Antipyrine salivary clearance was reduced to a similar extent (35%) in both cirrhotic groups compared to controls ($P < 0.01$). No difference was apparent between the two cirrhotic groups.

The metabolic clearances for production of the antipyrine metabolites are listed in Table 3. The metabolic clearance for 4-OH-antipyrine was not affected by cirrhosis. In contrast, the formation of 3-OH-antipyrine was reduced to a similar extent in both CCl_4- and bile duct ligation-induced cirrhosis (45 and 42%, respectively; $P < 0.05$). The formation of norantipyrine was most severely reduced in the CCl_4 group (65%; $P < 0.001$).

The cumulative urinary excretion of 3-OH-, nor- and 4-OH-antipyrine, and antipyrine in control animals averaged 23.9 ± 3.6, 8.8 ± 2.9, 14.3 ± 3.0 and 1.7 ± 0.3% of the dose, respectively. Urinary excretion of unchanged antipyrine was higher in CCl_4- and bile duct ligation-induced cirrhosis (3.5 ± 1.2 and 3.3 ± 1.0%, respectively; $P < 0.001$ versus controls).

The characteristics of the microsomal preparations are reported in Table 4. Microsomal protein content and cytochrome P450 were reduced in both cirrhotic groups. Microsomal cytochrome P450 content was more markedly reduced in the bile duct-ligated than in the CCl_4-cirrhotic rats ($P < 0.01$). The relative specific activities of NADPH-dependent cytochrome *c* reductase were similar in the three groups confirming that induction of cirrhosis did not invalidate the differential centrifugation procedure for harvesting microsomes [17, 23].

The formation of the three main metabolites as a function of antipyrine concentration by microsomes demonstrated saturation with increasing substrate concentration; all curves could adequately be described by Michaelis–Menten kinetics. Microsomes from the two cirrhotic groups exhibited similar kinetic behavior. The kinetic parameters describing the metabolite formation are reported in Table 5. V_{max} of 3-hydroxylation was decreased in a statistically significant fashion for bile duct-ligated rats only ($P < 0.05$), while the K_m was increased to a similar extent in both cirrhotic groups ($P < 0.05$). V_{max} and K_m of N-demethylation were increased in both cirrhotic groups compared to controls ($P < 0.01$), the increase being more pronounced in the bile duct-ligated than in the CCl_4-cirrhotic rats ($P < 0.05$). No effect of cirrhosis on 4-hydroxylation in microsomes could be demonstrated.

The intrinsic clearance derived from the enzyme kinetics (Table 5) and hepatic cytochrome P450 content (Table 4) are shown in Fig. 1. In both cirrhotic groups the intrinsic clearance of 3-OH- and norantipyrine was severely impaired ($P < 0.001$) while it was maintained for 4-OH-antipyrine. The reduction in intrinsic clearance was due to both the reduction of cytochrome P450 content (Table 4) and to the kinetic changes reported in Table 5.

The *in vivo* metabolic clearance of 3-OH-antipyrine correlated with its intrinsic clearance *in vitro* ($r = 0.658$, $P < 0.01$, see Fig. 2); this correlation was less convincing for norantipyrine ($r = 0.583$, $P < 0.01$) and absent for 4-OH-antipyrine (0.333, $P > 0.05$; data not shown).

The lipid composition of the microsomal fractions is given in Table 6. In both models of cirrhosis the cholesterol content of the microsomes was markedly increased compared to controls ($P < 0.001$). Sphingomyelin was increased in both cirrhotic groups while phosphatidylcholine, the major phospholipid, was reduced only in the bile duct-ligated cirrhotic rats ($p < 0.05$). The other individual and total phospholipid contents were not different between control and cirrhotic rats.

The ratio total phospholipids/cholesterol, a marker of membrane fluidity [37], was reduced in both cirrhotic groups (12.7 ± 5.6 in CCl_4 and 10.9 ± 4.5 in bile duct-ligated versus 27.6 ± 13.1 in controls; $P < 0.01$ and $P < 0.001$, respectively), indicating a more rigid microsomal membrane structure in cirrhotic animals. However, correlations between this parameter of fluidity and metabolite formation *in vitro* were poor, never exceeding 0.60; in contrast it correlated well with the *in vivo* clearance for production of norantipyrine ($r = 0.840$, see Fig. 3), but not for 3-OH- and 4-OH-antipyrine ($r = 0.599$ and 0.447, respectively; data not shown).

DISCUSSION

In the present investigation antipyrine metabolism was severely impaired in two models of cirrhosis;

Table 4. Characteristics of microsomal preparations obtained from control animals (CTR), cirrhosis induced by CCl_4/phenobarbital (CCl_4) and bile duct-ligated rats (BDL)

	Microsomal protein	Cytochrome P450	RSA
CTR	8.7 ± 1.9	1.07 ± 0.25	3.4 ± 1.1
CCl_4	5.7 ± 1.1	0.72 ± 0.35	4.3 ± 2.5
BDL	4.2 ± 0.7	0.31 ± 0.08	3.8 ± 0.8
Significance			
CTR vs CCl_4	<0.001	<0.01	NS*
CTR vs BDL	<0.001	<0.001	NS
CCl_4 vs BDL	<0.01	<0.01	NS

Microsomal protein is given in mg/g liver and cytochrome P450 in nmol/mg protein.
The relative specific activity (RSA) is the activity of NADPH-dependent cytochrome *c* reductase in microsomes divided by its activity in homogenate.
Means ± SD are given; the groups were compared by Peritz' F-test.
* Not significant by Peritz' F-test.

Table 5. Antipyrine metabolite formation *in vitro* by microsomal fractions from control animals (CTR), cirrhosis induced by CCl_4 (CCl_4) and bile duct-ligated rats (BDL)

	3-OH		NORA		4-OH	
	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m
CTR	2.5 ± 0.8	1.8 ± 0.2	1.8 ± 0.4	4.2 ± 1.3	1.3 ± 0.3	1.2 ± 0.6
CCl_4	2.0 ± 0.5	2.2 ± 0.6	2.8 ± 1.1	8.7 ± 4.7	1.8 ± 0.9	1.0 ± 0.7
BDL	1.6 ± 0.4	2.4 ± 0.5	11.4 ± 1.3	>20	1.2 ± 1.2	1.1 ± 0.9
Significance						
CTR vs CCl_4	NS*	<0.05	<0.01	<0.01	NS	NS
CTR vs BDL	<0.05	<0.05	<0.001	<0.001	NS	NS
CCl_4 vs BDL	NS	NS	<0.05	<0.05	NS	NS

V_{max} in nmol/min/nmol P450; K_m in mmol/L.
Means ± SD are given; the groups were compared by Peritz' F-test.
* Not significant by Peritz' F-test.

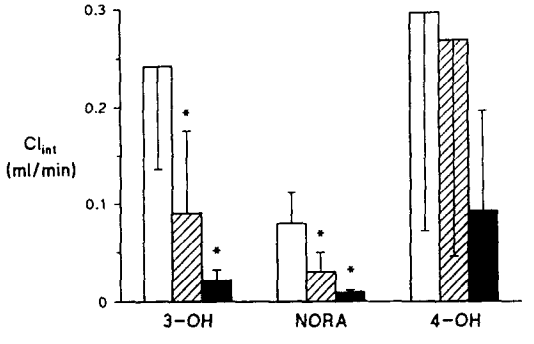

Fig. 1. Intrinsic clearances of antipyrine 3-hydroxylation (3-OH), 4-hydroxylation (4-OH) and N-demethylation (NORA) in control (□) rats and animals rendered cirrhotic by chronic exposure to phenobarbital/CCl_4 (▨) or bile duct ligation (■). Means ± SD are given. * Denotes a statistically significant difference from the control group as determined by Peritz' F-test.

this was mainly due to a reduced formation of 3-OH- and norantipyrine, while 4-hydroxylation was only minimally affected. The correlation between *in vivo* and *in vitro* metabolism was inconsistent: while there was a significant correlation between metabolic clearance *in vivo* of 3-OH-antipyrine and its intrinsic clearance *in vitro*, this was weak or non-existent for nor- and 4-OH-antipyrine. The ratio phospholipids/cholesterol of microsomes was decreased owing to increased cholesterol content in both models. This ratio, an indicator of membrane fluidity, correlated with the reduced metabolic clearance of norantipyrine but not with that of 3-OH- and 4-OH-antipyrine.

Our values of antipyrine metabolism *in vivo* and *in vitro* for control animals are in agreement with the literature [8, 24, 38]; similar to many studies in man [6, 9, 39] antipyrine clearance *in vivo* was markedly reduced in both rat models of liver cirrhosis studied. Although animals with biliary cirrhosis were more severely affected, the qualitative changes in metabolite pattern were similar in the two groups. 3-Hydroxylation was affected most severely, while changes in norantipyrine formation were less pronounced and 4-hydroxylation was not affected in a statistically significant fashion. This is different

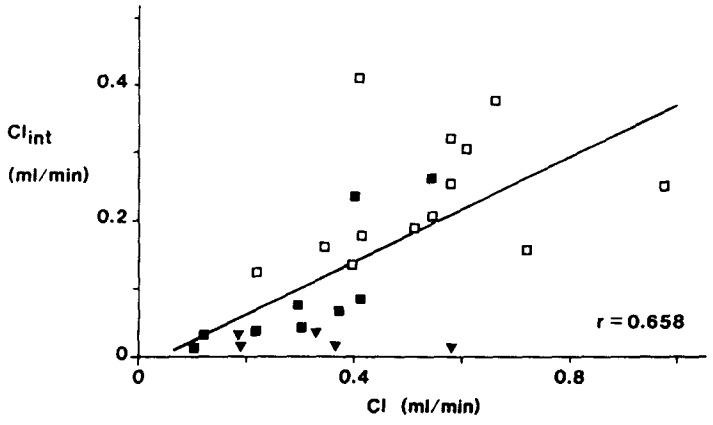

Fig. 2. Relationship between *in vitro* intrinsic clearance (Cl_{int}) and metabolic clearance *in vivo* of 3-OH-antipyrine of control animals (□), and rats with cirrhosis induced by treatment with phenobarbital/CCl_4 (■) or bile duct ligation (▼). The regression equation was $y = 0.388x - 0.015$ ($r = 0.658$, $P < 0.01$).

Table 6. Lipid composition of microsomal membranes from control animals, cirrhosis induced by CCl_4 (CCl_4) and bile duct-ligated rats (BDL)

	PL	XOL	SP	PC	SE	IN	PE
Controls	368 ± 118	15.4 ± 6.1	1.6 ± 0.8	77.5 ± 4.4	3.3 ± 1.2	9.1 ± 2.4	8.4 ± 2.8
CCl_4	350 ± 112	29.5 ± 6.2	2.9 ± 1.4	74.6 ± 6.1	4.8 ± 1.2	8.5 ± 1.7	9.8 ± 3.5
BBL	385 ± 92	39.8 ± 14.2	3.9 ± 1.4	70.3 ± 6.5	4.0 ± 1.6	8.5 ± 4.2	11.4 ± 1.3
Significance							
Controls vs CCl_4	NS*	<0.001	<0.05	NS	<0.05	NS	NS
Controls vs BDL	NS	<0.001	<0.001	<0.05	NS	NS	NS
CCl_4 vs BDL	NS	NS	NS	NS	NS	NS	NS

Phospholipids and cholesterol are given in μg/mg protein, the individual phospholipids in % of total phospholipids. Means ± SD are given; the groups are compared by Peritz' F-test.

PL, total phospholipids; XOL, cholesterol; SP, sphingomyeline; PC, phosphatidylcholine; SE, phosphatidylserine; IN, phosphatidylinositol; PE, phosphatidylethanolamine.

* Not significant by Peritz' F-test.

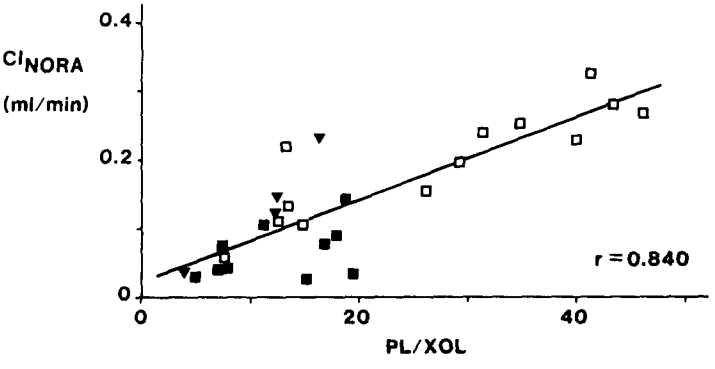

Fig. 3. Relationship between metabolic clearance of norantipyrine (Cl_{NORA}) *in vivo* and the ratio of total phospholipids/cholesterol (PL/XOL), a measure of membrane fluidity, in microsomal membranes of control animals (□), and rats with cirrhosis induced by treatment with phenobarbital and CCl_4 (■) or bile duct ligation (▼). The regression equation was $y = 0.00599x + 0.023$ ($r = 0.840$, $P < 0.01$).

from alcoholic cirrhosis in man, where a decrease in 4-hydroxylation is compensated by an increase in 3-hydroxylation [6], but similar to an acute toxicity study in rats where 4-hydroxylation was spared [40].

This differential effect of cirrhosis on the different metabolic pathways of antipyrine could be related to different susceptibility of isoenzymes of cytochrome P450 to hepatotoxins as shown in other studies [6, 41]. Alternatively, the lipid changes described in the present study could be responsible for these effects (see below). Metabolism of antipyrine and aminopyrine was affected to a variable extent in cirrhotic animals (Tables 1 and 2); thus, with control animals a continuum is obtained which is well suited to the study of factors that affect antipyrine metabolism [17, 23, 40, 42].

The *in vivo* metabolic clearance correlated with the intrinsic clearance *in vitro* for 3-OH-antipyrine only (Fig. 3). A similar *in vivo–in vitro* correlation has been shown in humans [9]; however, in the present experiments this correlation ($r = 0.658$) explains only 43% of the data. This suggests that other factors are more important in predicting impaired clearance of xenobiotics in chronic liver disease. We have previously demonstrated that the loss of hepatocyte mass [22, 23, 43] and alterations of hepatic microcirculation [43] can explain up to 80% of the variation in the clearance of low extraction compounds in rat models of cirrhosis.

For norantipyrine and 4-OH-antipyrine this lack of correlation could be due to further metabolism since in rats about 50–65% of the dose is excreted as 3-OH-, nor- and 4-OH-antipyrine [5], but over 90% of a radioactive dose of antipyrine is recovered in urine [44, 45]. Indeed, double oxidation of 4-OH- and norantipyrine has been reported [29]; this could obscure a better *in vivo–in vitro* correlation [8]. Reduction of double oxidation products of 4-OH-antipyrine in cirrhotic rats could also explain the observed lack of change in metabolic clearance for 4-OH-antipyrine.

In both models of cirrhosis the cholesterol content of the microsomal fractions was increased (Table 6). The increase of cholesterol in the bile duct-ligated cirrhotic group is in agreement with previous work by other investigators [14–16, 46] and our own group [17, 47]. Total phospholipid content was not affected in either cirrhosis model. In both experimental groups sphingomyelin was significantly decreased; in addition, phosphatidylcholine was selectively decreased in the bile duct-ligated cirrhotic rats. An increase in both cholesterol and sphingomyelin content conveys increased rigidity to microsomal membranes [37]; we have previously demonstrated this using diphenyl hexatriene fluorescence polarization [17]. The ratio of total phospholipids/cholesterol can be used as a measurement of membrane fluidity [37]. Decreased fluidity is thought to interfere with the interaction between NADPH-dependent cytochrome *c* reductase and cytochrome P450 in some but not all instances [13, 48]. Although no correlation existed between these lipid alterations and antipyrine metabolism *in vitro*, the decreased affinity (see K_m in Table 5) observed for nor- and 3-OH-antipyrine formation could be due to a lipid effect [11, 48].

In contrast to the *in vitro* data there was an excellent correlation between the phospholipid/cholesterol ratio and the *in vivo* metabolic clearance of norantipyrine (Fig. 3). The reasons for this discrepancy are unclear but we have recently observed a similar correlation between aminopyrine N-demethylation *in vivo* and decreases in microsomal membrane fluidity [17].

In conclusion, the isoenzymes responsible for antipyrine oxidation are affected to different extents by cirrhosis while the etiology of cirrhosis does not appear to have a major effect in the rat. Intrinsic clearance predicts only *in vivo* metabolism of 3-hydroxylation; this correlation explains only about 40% of the variability, however, pointing to the fact that other factors are important determinants of xenobiotic metabolism in cirrhosis. Among them lipid composition of the microsomal membrane may play a role in some pathways of antipyrine metabolism but the loss of hepatocytes and changes in microcirculation could be more important determinants of xenobiotic metabolism in cirrhosis.

Acknowledgements—This study was supported by SNF grants 32.9365.87 and 32.30168.90; J. T. M. Buters was partly supported by the Berne Liver Foundation. The HPLC equipment was purchased with a grant from the Sandoz Foundation. This study would not have been possible without the expert technical assistance of Ms C. Talos and Mr H. Sägesser and the careful animal work of Mr K. Krusch. The artwork of M. Kappeler and the help of B. Oeschger in preparing the manuscript are gratefully appreciated.

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