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REGULATION OF RENAL CYTOCHROME P-450

EFFECTS OF TWO-THIRDS HEPATECTOMY, CHOLESTASIS, BILIARY CIRRHOSIS AND POST-NECROTIC CIRRHOSIS ON HEPATIC AND RENAL MICROSOMAL ENZYMES

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Abstract—The possibility of a relationship between hepatic and renal cytochrome P-450 contents was assessed in rats with liver disease. In rats killed 3 days after two-thirds hepatectomy (a model for hepatocellular insufficiency), the total microsomal cytochrome P-450 content of the whole liver was decreased by 60% as compared to that in control rats; renal cytochrome P-450 was increased by 30% while the 7-ethoxycoumarin deethylase activity of kidney microsomes was increased by 80%. In rats killed 7 days after bile duct ligation (a model for cholestasis) or 35 days after bile duct ligation (a model for biliary cirrhosis), hepatic cytochrome P-450 was decreased by 60% and 45%, respectively, while renal cytochrome P-450 content was increased by 50% and 150%, respectively. In contrast, in rats killed 15 days after the last dose of carbon tetrachloride, 1.3 ml/kg twice weekly for 3 months (a model for post-necrotic cirrhosis), both hepatic and renal cytochrome P-450 contents remained unchanged. Phenobarbital (80 mg/kg daily for 3 days) was a poor inducer of renal cytochrome P-450 in sham-operated rats but became a potent inducer of renal cytochrome P-450 in rats with two-thirds hepatectomy. We conclude that renal cytochrome P-450 is increased in three models in which hepatic cytochrome P-450 contents are decreased (two-thirds hepatectomy, cholestasis and biliary cirrhosis), but remains unchanged in a model of severe liver pathology, in which hepatic cytochrome P-450 content is not modified (late, post-necrotic cirrhosis). The hypothetical role of endogenous inducer(s) is discussed.

Cytochrome P-450 functions as the terminal oxidase in a microsomal monooxygenase system which metabolizes both xenobiotics and endogenous compounds such as fatty acids, prostaglandins, sex steroids, cholesterol, bile acids or vitamin D [1–5]. Cytochrome P-450 is most abundant in the liver where it is made up of several isozymes with different (albeit overlapping) substrate specificities [6–8]. Cytochrome P-450 is also present in extrahepatic organs such as kidney and lung; attempts to isolate and/or characterize the cytochrome P-450 isozymes present in these extrahepatic organs are just beginning [9–15]. Nevertheless, from the different activity of microsomes from various organs with different substrates, it is clear that the cytochrome P-450 isozymes which are present in kidney and lung must differ, at least in proportion, and also perhaps in nature (for some isozymes), from those present in the liver.

In the liver, the concentration of each cytochrome P-450 isozyme is genetically determined; furthermore, there are built-in genetic regulatory mechanisms allowing for the synthesis of the mRNAs of several isozymes to be increased by the administration of exogenous inducers [16–20]. Interestingly, some endogenous substrates (or products) may also interact with such regulatory genetic mechanisms. For example, cholesterol 7α-hydroxylase, an activity mediated by a hepatic cytochrome P-450 isozyme, is the rate limiting step in the synthesis of bile acids from cholesterol [21]; this activity is markedly enhanced when the bile acid pool is depleted by administration of cholestyramine or the creation of a bile fistula [22]. Hepatic cytochrome P-450 isozymes may be modulated also by the administration of endogenous substrates such as cholesterol, sex steroids or nicotinamide [23–27]. Interestingly, endogenous substrates may also regulate renal cytochrome P-450. Thus, renal cytochrome P-450 is highly active in the ω-oxidation of laurate [28]; administration of this endogenous substrate markedly increased renal cytochrome P-450 as well as the type I binding spectrum of laurate and its ω-oxidation [29].

From the above-mentioned observations, it is tempting to speculate that the "basal" concentration of several cytochrome P-450 isozymes is determined, first, by the genetic framework of the individual, and in addition, by the influence of endogenous inducers (or repressors) on this particular genetic machinery. A corollary of this hypothesis might be that levels of cytochrome P-450 in the liver might indirectly influence the levels of at least some cytochrome P-450 isozymes in extrahepatic organs. For example, a decreased cytochrome P-450 content in the liver may decrease the overall metabolism of an endogenous

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Table 1. Effects of partial hepatectomy on microsomal enzymes

	Organ weight (g)	Microsomal protein (mg/g organ)	NADPH-cytochrome c reductase (nmole/min/mg protein)	NADPH-cytochrome c reductase (μmole/min/whole organ)	Cytochrome P-450 (nmole/mg protein)	Cytochrome P-450 (nmole/whole organ)
Liver						
Control rats	10 ± 1	55 ± 10	35 ± 9	19 ± 4	0.58 ± 0.14	329 ± 48
Sham-operated rats	10 ± 1	56 ± 5	28 ± 9	16 ± 5	0.51 ± 0.09	284 ± 59
Hepatectomized rats	6.5 ± 0.9*	54 ± 5	30 ± 9	10 ± 3*	0.39 ± 0.06	135 ± 25*
Kidneys						
Control rats	2.1 ± 0.1	29 ± 5	7 ± 5	0.42 ± 0.15	0.11 ± 0.04	6.6 ± 1.6
Sham-operated rats	2.2 ± 0.3	28 ± 4	10 ± 4	0.51 ± 0.17	0.13 ± 0.02	7.3 ± 1.5
Hepatectomized rats	2.1 ± 0.2	27 ± 2	11 ± 3	0.59 ± 0.16	0.15 ± 0.03*	8.5 ± 1.3*
Lungs						
Control rats	1.2 ± 0.3	13 ± 4	13 ± 2	0.21 ± 0.10	0.023 ± 0.011	0.34 ± 0.15
Sham-operated rats	1.2 ± 0.3	12 ± 4	14 ± 3	0.20 ± 0.08	0.018 ± 0.007	0.27 ± 0.12
Hepatectomized rats	1.1 ± 0.1	14 ± 4	14 ± 3	0.21 ± 0.10	0.017 ± 0.007	0.27 ± 0.14

Rats were killed 3 days after laparatomy (sham-operated rats) or two-thirds hepatectomy (hepatectomized rats). Results are means ± S.D. for 12 rats. Kidney and lung data are for the 2 kidneys and the 2 lungs.
* Significantly different from that in sham-operated rats (*t*-test for independent data), $P < 0.05$.

inducer, increase its steady state concentration in the body, and thereby induce some cytochrome P-450 isozyme(s) in the kidney.

Testing for this corollary may prove difficult, however. Although many chemicals are known that would decrease or increase hepatic cytochrome P-450 contents, such chemicals may also exert, to some extent, their effects in extrahepatic organs. In an attempt to overcome this difficulty, we have studied several models in which hepatic cytochrome P-450 contents are decreased through surgical procedures: partial hepatectomy, a model for hepatocellular insufficiency [30, 31], bile duct ligation for 7 days, a model for cholestasis [32–35], and bile duct ligation for 35 days, a model for biliary cirrhosis [36]. As a "control" situation, we have also studied a model of late post-necrotic cirrhosis [37], in which there is marked liver pathology but normal hepatic cytochrome P-450 contents [38].

MATERIALS AND METHODS

Animals, surgical procedures and treatments. Male Sprague–Dawley rats, Crl:CD ^{R}(SD) BR, weighing 180–220 g, were purchased from Charles River, France (St-Aubin-les-Elbeuf, France). Rats were fed *ad libitum* with a normal standard diet (Autoclavé 113, UAR, France).

Some rats were laparotomized (sham). Other rats underwent a two-thirds hepatectomy as described by Higgins and Anderson [31]. In other rats, the bile duct was ligated in 2 places, 1 cm apart, with non-resorbable sutures, and then resected in between, as reported by Franco *et al.* [36].

Some of the hepatectomized rats and their sham-operated controls received phenobarbital, 80 mg/kg i.p. daily for 3 days, the first dose of the barbiturate being given 5 hr after completion of the surgical procedure; rats were killed 19 hr after the last dose of the inducer.

Other rats received carbon tetrachloride, 1.3 ml/kg i.p., mixed with an equal volume of corn oil, twice weekly, for 3 months; rats were killed 15 days after the last dose of the hepatotoxin.

Preparation of microsomes. Rats were stunned and laparotomized. To remove red cells and their hemoglobin, we perfused kidneys and lungs *in situ* with ice-cold 0.154 M NaCl. Kidneys were perfused through the inferior vena cava clamped above, and under, the renal veins; lungs were perfused through the upper part of the inferior vena cava clamped above the hepatic veins. The liver was not perfused. Liver, kidneys and lungs were removed, washed in ice-cold saline, gently expressed (kidneys and lungs), blotted dry, weighed, minced and homogenized in 3 volumes of 0.154 M KCl, 0.01 M sodium–potassium phosphate buffer, pH 7.4.

The tissue homogenates were centrifuged at 10,000 *g* for 30 min; the 10,000 *g* supernatants were then centrifuged at 100,000 *g* for 60 min. Liver and kidney microsomal pellets were stored at −20°. Lung microsomes were further washed by resuspending the microsomal pellets in buffer and centrifuging again at 100,000 *g*. The washed pellets were then resuspended in 0.1 M sodium–potassium phosphate

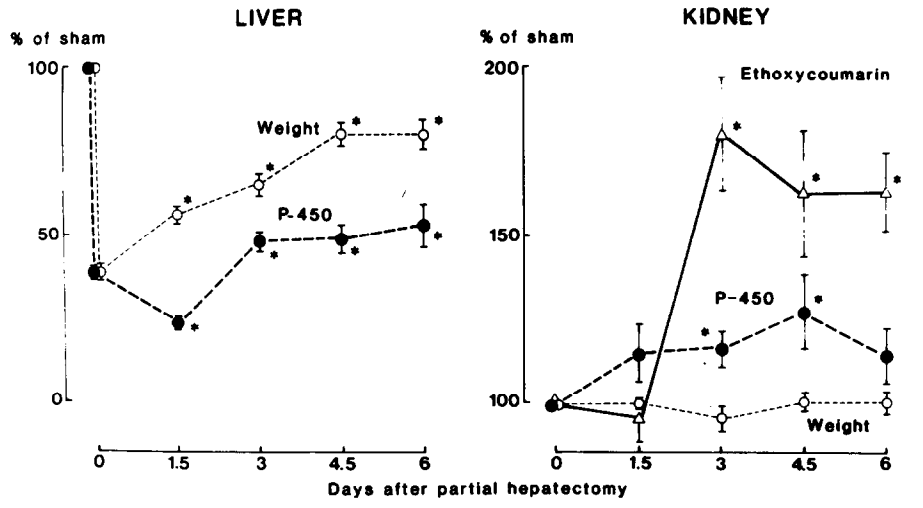

Fig. 1. Time course of the effects of two-thirds hepatectomy on hepatic and renal microsomal enzymes. Rats were killed at various times after partial hepatectomy or laparotomy ("sham"). The figure shows various parameters (organ weight, total microsomal cytochrome P-450 content of the whole organ, and 7-ethoxycoumarin deethylase activity of kidney microsomes) expressed as per cent of the mean value in simultaneously killed sham-operated rats. Results are means ± S.E.M. for 12 rats. The asterisks indicate a significant difference from values in sham-operated rats (*t*-test for independent data), $P < 0.05$.

buffer, pH 7.4 containing 20% glycerol, and were stored at −20°.

Microsomal enzymes. Microsomal protein content was measured by the method of Lowry *et al.* [39]. Liver microsomal cytochrome P-450 was measured by the technique of Omura and Sato [40] with microsomal suspensions containing microsomes from 62 mg of liver per ml (in 0.05 M sodium–potassium phosphate buffer, pH 7.4 containing 1 mM EDTA). Kidney microsomal cytochrome P-450 was measured with similarly prepared microsomal suspensions, by the method described by Jacobsson and Cinti [41]: the microsomal suspension was divided into 2 cuvettes; 1 mM succinate was added in both cuvettes and CO was bubbled in both cuvettes for 30 sec; the base line was then recorded on an SLM-Aminco DW-2C spectrophotometer. Dithionite was then added in the sample cuvette and the CO-binding spectrum was recorded, with base line subtraction.

Lung microsomal cytochrome P-450 was measured according to the technique of Matsubara *et al.* [42]. The microsomal suspension containing microsomes from 62 mg of lung per ml was divided into 2 cuvettes; 1 mM NADH was added in both cuvettes and the base line was recorded. Dithionite was added, and CO was bubbled, in the sample cuvette; the CO-binding spectrum was then recorded with base line subtraction.

Kidney microsomal heme and cytochrome b_5 were measured as described by Omura and Sato [40], with suspensions containing microsomes from 62 mg of kidney per ml.

NADPH-cytochrome *c* reductase activity was measured as described by Mazel [43] with either microsomes or the 100,000 *g* supernatant from 12 mg of liver, 50 mg of kidney, or 62 mg of lung.

Binding spectra and monooxygenase activities. Kidney microsomes were resuspended in 0.154 M KCl, 0.01 M sodium–potassium phosphate buffer, pH 7.4 and centrifuged again at 100,000 *g* for 60 min. Microsomal suspensions containing 1.5 mg of microsomal protein per ml were prepared in the same buffer, and divided into 2 cuvettes. After recording of the base line, laurate (4 mM) was added, in 80 μl of methanol, to the sample cuvette while a same volume of methanol was added to the reference cuvette. The binding spectrum was recorded, with base line subtraction, from 330 to 480 nm, on a SLM-Aminco DW-2C spectrophotometer.

To record the binding spectrum of vitamin D_3, we had to eliminate the absorption of the vitamin itself. Therefore, cuvettes divided into 2 compartments were used. The microsomal suspension was placed in one compartment of both cuvettes, while the buffer solution was placed in the other compartment. After recording the base line, vitamin D_3 (4 mM) was added, in 40 μl of methanol, to the microsomal suspension in the sample cuvette and to the buffer solution in the reference cuvette; the same volume of methanol was added to the buffer solution in the sample cuvette and to the microsomal suspension in the reference cuvette; the binding spectrum was then recorded.

The demethylation of aminopyrine (1.7 mM) was measured by the technique of Mazel [43], with kidney microsomes from 250 mg of kidney, in the presence of the following NADPH-generating system: NADP (0.2 mM), glucose-6-phosphate (3.3 mM), and glucose-6-phosphate dehydrogenase (5 enzyme units/ml). The hydroxylation of benzo(*a*)pyrene (0.2 mM) was measured as reported by Kuntzman *et al.* [44] with kidney microsomes from 50 mg of kidney, in the presence of the same NADPH-generating system. The deethylation of 7-ethoxycoumarin (0.4 mM) was measured as reported by Greenlee and Poland [45], with kidney microsomes from 50 mg

Table 2. Effects of partial hepatectomy on binding spectra and monooxygenase activities in kidney microsomes

	Amplitude of binding spectra		Monooxygenase activities		
	Laurate (type I) (O.D. × 10^3/mg protein per ml)	Vitamin D_3 (reverse type I) (O.D. × 10^3/mg protein per ml)	7-Ethoxycoumarin	Aminopyrine (nmole/min/mg protein)	Benzo(*a*)pyrene
Sham-operated rats	0.35 ± 0.08	3.3 ± 0.7	0.010 ± 0.004	0.31 ± 0.10	0.002 ± 0.002
Hepatectomized rats	0.35 ± 0.11	3.1 ± 0.8	0.018 ± 0.006*	0.29 ± 0.07	0.003 ± 0.002

Rats were killed 3 days after laparotomy (sham-operated rats) or two-thirds hepatectomy (hepatectomized rats). Results are means ± S.D. for 12 rats, O.D., optical density.

* Significantly different from that in sham-operated rats (*t*-test for independent data), $P < 0.05$.

Table 3. Inducing effects of phenobarbital on liver and kidney microsomal enzymes in sham-operated and hepatectomized rats

	Organ weight (g)	Microsomal protein (mg/g organ)	NADPH–cytochrome *c* reductase		Cytochrome P-450	
			(nmole/min/mg protein)	(μmole/min/whole organ)	(nmole/mg protein)	(nmole/whole organ)
Liver						
Sham-operated, untreated	10 ± 1	57 ± 5	31 ± 11	17 ± 6	0.55 ± 0.08	309 ± 63
Sham-operated, phenobarbital-treated	12 ± 1*	62 ± 4	53 ± 8*	39 ± 8*	1.11 ± 0.16*	809 ± 158*
Hepatectomized, untreated	6.0 ± 0.7	54 ± 6	33 ± 11	10 ± 4	0.41 ± 0.05	132 ± 24
Hepatectomized, phenobarbital-treated	5.4 ± 0.7	67 ± 4*	47 ± 7*	16 ± 3*	0.81 ± 0.29*	272 ± 84*
Kidneys						
Sham-operated, untreated	1.9 ± 0.2	34 ± 5	8.0 ± 4.6	0.52 ± 0.27	0.10 ± 0.02	6.5 ± 1.0
Sham-operated, phenobarbital-treated	2.0 ± 0.2	39 ± 3*	9.1 ± 2.7	0.73 ± 0.27	0.10 ± 0.03	8.2 ± 2.6*
Hepatectomized, untreated	1.9 ± 0.2	39 ± 6	9.1 ± 3.1	0.67 ± 0.24	0.12 ± 0.03	9.0 ± 1.5
Hepatectomized, phenobarbital-treated	1.9 ± 0.2	42 ± 5	11.0 ± 3.9	0.85 ± 0.30	0.18 ± 0.05*	14.0 ± 3.9*

Rats were killed 3 days after laparotomy (sham-operated) or two-thirds hepatectomy (hepatectomized). Some rats received phenobarbital, 80 mg/kg i.p. daily for 3 days, starting 5 hr after surgery. Results are means ± S.D. for 6 rats. Kidney data are for the 2 kidneys.

* Significantly different from that in rats not receiving phenobarbital (*t*-test for independent data), $P < 0.05$.

Table 4. Effects of biliary cirrhosis (bile duct ligation for 35 days) on microsomal heme, cytochrome b_5 and cytochrome P-450

	Organ weight (g)	Microsomal protein (mg/g organ)	Heme (nmole/ mg protein)	Cytochrome b_5 (nmole/ mg protein)	Cytochrome P-450 (nmole/ mg protein)	Cytochrome P-450 (nmole/ whole organ)
Liver						
Sham-operated rats	15 ± 2	35 ± 4	1.58 ± 0.25	1.11 ± 0.30	0.65 ± 0.09	335 ± 83
Rats with biliary cirrhosis	22 ± 6*	31 ± 9	0.99 ± 0.28*	0.67 ± 0.37*	0.27 ± 0.07*	185 ± 103*
Kidneys						
Sham-operated rats	2.7 ± 0.4	21 ± 7	0.79 ± 0.22	0.54 ± 0.28	0.12 ± 0.05	6.6 ± 1.8
Rats with biliary cirrhosis	2.7 ± 1.4	20 ± 6	1.10 ± 0.23*	0.58 ± 0.26	0.27 ± 0.17*	16.2 ± 11.4*
Lungs						
Sham-operated rats	1.5 ± 0.2	14 ± 4	1.21 ± 0.13	0.81 ± 0.17	0.019 ± 0.008	0.39 ± 0.21
Rats with biliary cirrhosis	1.9 ± 0.4	17 ± 5*	1.50 ± 0.32	0.98 ± 0.29	0.017 ± 0.001	0.52 ± 0.18*

Rats were killed 35 days after laparotomy (sham-operated) or bile duct ligation; only those of the latter rats which had micronodular livers were used (rats with biliary cirrhosis). Results are means ± S.D. for 24 rats. Kidney and lung data are for the 2 kidneys and the 2 lungs.
* Significantly different from that in sham-operated rats (*t*-test for independent data), $P < 0.05$.

of kidney, in the presence of the same NADPH-generating system.

Bile acid concentrations and in vitro *effects of bile acids on microsomal enzymes.* Livers, kidneys and lungs were homogenized in 10 volumes of ethanol. The homogenates were centrifuged at 5000 *g* and the supernants were saved. The extraction procedure was repeated twice. The pooled ethanol extracts were combined and evaporated. The residue was taken up in methanol. The concentration of bile acids in tissue extracts and in serum were determined as described by Nicholas *et al.* [46].

The binding spectra of laurate and cholecalciferol, measured with pooled kidney microsomes from control rats, were measured again after addition of various bile acids (1 mM) to the microsomal suspension. Monooxygenase activities of pooled kidney microsomes from control rats were also measured in the presence of various bile acids (1 mM).

The *in vitro* solubilizing effects of bile acids on kidney microsomal cytochrome *c* reductase was determined as follows. A microsomal suspension containing microsomes from 100 mg of kidney per ml was prepared. Part of the ice-cold microsomal suspension received no addition; in other aliquots, various bile acids (1 mM) were added. After 60 min, the suspensions were centrifuged at 100,000 *g* for 60 min; microsomal pellets were resuspended in buffer. NADPH cytochrome *c* reductase activity and cytochrome P-450 were then measured both in the resuspended microsomes and in the 100,000 *g* supernatants.

Histology. Tissue fragments were placed in Bouin's fluid; they were embedded in paraffin 24 hr later, cut and stained with hematoxylin and eosin.

RESULTS

Partial hepatectomy

As expected, two-thirds hepatectomy markedly decreased the cytochrome P-450 content and the NADPH-cytochrome *c* reductase activity of the whole liver (Table 1, Fig. 1). In contrast, it increased cytochrome P-450 content in the kidney (Table 1, Fig. 1). There was also a trend for a higher NADPH cytochrome *c* reductase of kidney microsomes (Table 1). The 7-ethoxycoumarin deethylase activity of renal microsomes was increased by 80% (Fig. 1, Table 2), but other renal monooxygenase activities or binding spectra were not significantly modified (Table 2). Microsomal enzymes were unchanged in lung (Table 1).

Phenobarbital (80 mg/kg daily for 3 days) increased renal cytochrome P-450 by only 26% in sham-operated rats, but further increased it by 55% in hepatectomized rats (Table 3). This daily dose of phenobarbital produced marked sleepiness in the hepatectomized animals, while higher doses (100 mg/kg daily) resulted in coma and several deaths.

Biliary cirrhosis

After 35 days of bile duct ligation, 63% of the animals had nodular livers and were used in this study. Histological examination of these livers showed portal and periportal fibrosis, ductular pro-

Table 5. Effects of biliary cirrhosis (bile duct ligation for 35 days) on microsomal, cytosolic and total NADPH–cytochrome *c* reductase activity

	NADPH–cytochrome *c* reductase activity		
	Microsomal	Cytosolic (μmole/min/whole organ)	Total
Liver			
Sham-operated rats	19 ± 4	0.71 ± 0.32	20 ± 4
Rats with biliary cirrhosis	13 ± 6*	1.48 ± 0.35*	15 ± 6
Kidneys			
Sham-operated rats	0.62 ± 0.23	0.52 ± 0.19	1.1 ± 0.4
Rats with biliary cirrhosis	0.48 ± 0.18	1.10 ± 0.51*	1.6 ± 0.6*
Lungs			
Sham-operated rats	0.41 ± 0.08	0.024 ± 0.016	0.43 ± 0.07
Rats with biliary cirrhosis	0.72 ± 0.24*	0.051 ± 0.044	0.77 ± 0.26*

Rats were killed 35 days after laparotomy (sham-operated rats) or bile duct ligation; only those rats with micronodular livers were used (rats with biliary cirrhosis). The reductase activity was measured both in microsomes (microsomal activity) and in the 100,000 *g* supernatant fraction (cytosolic activity). The total activity was calculated as the sum of the activity in microsomes and that in cytosol. Results are means ± S.D. for 16 rats. Kidney and lung data are for the 2 kidneys and the 2 lungs.

* Significantly different from that in sham-operated rats (*t*-test for independent data), $P < 0.05$.

liferation and disorganization of the hepatic architecture. Kidneys and lungs were histologically normal.

Biliary cirrhosis decreased by 45% the microsomal cytochrome P-450 content of the whole liver (Table 4), but increased it by 150% in the kidney and by 30% in lung (Table 4). The increased renal cytochrome P-450 content was corroborated by an increase in total heme, while cytochrome b_5 remained unchanged (Table 4).

The total activity of NADPH–cytochrome *c* reductase (microsomal + cytosolic) was modified as was microsomal cytochrome P-450: it tended to decrease in the liver but was increased in kidney and lung (Table 5). However, cholestasis modified the repartition of NADPH–cytochrome *c* reductase, the cytosolic activity being increased in all organs (Table 5). The microsomal NADPH–cytochrome *c* reductase activity was decreased in liver, tended to decrease in kidney and was increased in lung (Table 5). The binding spectra of laurate and vitamin D_3 as well as tested monooxygenase activities (7-ethoxycoumarin, aminopyrine and benzo(*a*)pyrene) were unchanged in kidney microsomes (not shown).

Cholestasis

After 7 days of bile duct ligation, the livers were increased in size but were not micronodular. Histologic examination showed cholestasis, ductular proliferation, but neither fibrosis nor disorganization of the hepatic architecture.

As in the preceding model, microsomal cytochrome P-450 content was markedly decreased in the liver (Table 6). The effects on renal microsomal enzymes were similar to, but weaker than, those after 35 days of bile duct ligation. Indeed, kidney microsomal cytochrome P-450 was increased by only 50% (Table 6). As in the preceding model, NADPH–cytochrome *c* reductase activity tended to decrease in kidney microsomes (Table 6), while the cytosolic activity and the total activity (microsomal + cytosolic) were increased by 80% and 44% respectively (not shown). Tested binding spectra (laurate and vitamin D_3) or monooxygenase activities (7-ethoxycoumarin, aminopyrine, and benzo(*a*)pyrene) remained unchanged in kidney microsomes (not shown).

Lung microsomal enzymes were not modified (Table 6).

Postnecrotic cirrhosis

Rats were killed 15 days after the last dose of carbon tetrachloride; 64% of the animals had micronodular livers and were used in this study. Histologic examination of these livers showed extensive fibrosis and regeneration nodules; at that time, there was no necrosis and no steatosis. Kidneys and lungs were histologically normal.

The hepatic content of cytochrome P-450 remained unchanged, and kidney and lung microsomal enzymes were not modified (Table 7).

Bile acid concentrations and in vitro *effects of bile acids on microsomal enzymes*

After short or prolonged bile duct ligation, bile acids were increased in serum, kidney and lung (Table 8); in the liver, bile acids were markedly increased after 7 days of bile duct ligation but not after 35 days (Table 8). After two-third hepatectomy, bile acids were slightly increased in serum, but remained unchanged in tissues (Table 8). Bile acid levels were not measured in the model of postnecrotic cirrhosis.

In vitro, addition of several bile acids (1 mM) to kidney microsomes markedly decreased the activity of 7-ethoxycoumarin deethylase (Table 9); however, the demethylation of aminopyrine and the binding spectra of laurate and vitamin D_3 were not modified (Table 9).

In vitro, addition of several bile acids (1 mM) to resuspended kidney microsomes (followed by recentrifugation and measurement of cytochrome P-450 and NADPH–cytochrome *c* reductase both in the microsomal fraction and in the 100,000 *g* supernatant

Table 6. Effects of cholestasis (bile duct ligation for 7 days) on microsomal enzymes

	Organ weight (g)	Microsomal protein (mg/g organ)	NADPH–cytochrome *c* reductase		Cytochrome P-450	
			(nmole/min/ mg protein)	(μmole/min/ whole organ)	(nmole/ mg protein)	(nmole/ whole organ)
Liver						
Control rats	12 ± 2	42 ± 8	78 ± 31	38 ± 14	0.66 ± 0.08	325 ± 30
Sham-operated rats	12 ± 2	33 ± 8	87 ± 34	31 ± 11	0.68 ± 0.12	250 ± 53
Rats with cholestasis	14 ± 1*	30 ± 7	67 ± 27	26 ± 9	0.35 ± 0.10*	140 ± 32*
Kidneys						
Control rats	2.3 ± 0.2	26 ± 5	15 ± 8	0.95 ± 0.62	0.09 ± 0.03	5.5 ± 1.9
Sham-operated rats	2.2 ± 0.1	27 ± 5	16 ± 8	0.96 ± 0.62	0.09 ± 0.02	5.5 ± 1.9
Rats with cholestasis	2.2 ± 0.3	25 ± 5	12 ± 9	0.71 ± 0.59	0.15 ± 0.02*	8.5 ± 2.3*
Lungs						
Control rats	1.3 ± 0.1	13 ± 1	28 ± 12	0.41 ± 0.15	0.018 ± 0.006	0.30 ± 0.10
Sham-operated rats	1.2 ± 0.1	14 ± 3	29 ± 9	0.46 ± 0.12	0.010 ± 0.006	0.24 ± 0.12
Rats with cholestasis	1.4 ± 0.5	13 ± 4	30 ± 16	0.48 ± 0.24	0.017 ± 0.008	0.32 ± 0.24

Rats were killed 7 days after laparotomy (sham-operated rats) or bile duct ligation (rats with cholestasis). Results are means ± S.D. for 12 rats. Kidney and lung data are for the 2 kidneys and the 2 lungs.

* Significantly different from that in sham-operated rats (*t*-test for independent data), $P < 0.05$.

Table 7. Effects of late, post-necrotic cirrhosis on microsomal enzymes

	Organ weight (g)	Microsomal protein (mg/g organ)	NADPH–cytochrome *c* reductase		Cytochrome P-450	
			(nmole/min/ mg protein)	(μmole/min/ whole organ)	(nmole/ mg protein)	(nmole/ whole organ)
Liver						
Control rats	19 ± 2	57 ± 10	29 ± 4	30 ± 8	0.49 ± 0.07	525 ± 64
Rats with post-necrotic cirrhosis	21 ± 4	54 ± 6	35 ± 5	40 ± 18	0.49 ± 0.05	538 ± 173
Kidneys						
Control rats	3.2 ± 0.2	42 ± 5	7.7 ± 0.8	1.0 ± 0.2	0.10 ± 0.04	13 ± 6
Rats with post-necrotic cirrhosis	3.2 ± 0.5	39 ± 8	7.6 ± 1.4	0.9 ± 0.2	0.10 ± 0.02	12 ± 4
Lungs						
Control rats	1.5 ± 0.2	9 ± 2	17 ± 2	0.23 ± 0.04	0.024 ± 0.017	0.32 ± 0.24
Rats with post-necrotic cirrhosis	1.6 ± 0.3	8 ± 2	16 ± 4	0.21 ± 0.07	0.022 ± 0.021	0.30 ± 0.29

Rats received CCl_4, 1.3 ml/kg i.p. in an equal volume of corn oil, twice weekly for 3 months; rats were killed 15 days after the last dose of CCl_4; only those rats with micronodular livers were used (rats with post-necrotic cirrhosis). Other rats received corn oil and were used 15 days after the last dose of corn oil (control rats). Results are means ± S.D. for 8 rats. Kidney and lung data are for the 2 kidneys and the 2 lungs.

Table 8. Effects of partial hepatectomy or bile duct ligation on bile acid concentrations

	Total bile acids			
	Serum (nmole/ml)	Liver (nmole/g)	Kidney (nmole/g)	Lung (nmole/g)
Control	9 ± 4	450 ± 160	33 ± 11	11 ± 4
Partial hepatectomy	81 ± 4*	660 ± 180	50 ± 28	19 ± 10
Bile duct ligation for 7 days	1900 ± 560*	2000 ± 370*	83 ± 30*	120 ± 51*
Bile duct ligation for 35 days	260 ± 110*	510 ± 100	240 ± 60*	96 ± 36*

Rats were killed 3 days after two-thirds hepatectomy, and 7 or 35 days after bile duct ligation; in the latter group, only those rats with micronodular livers were used. Results are means ± S.D. for 4–9 rats.
* Significantly different from that in control rats (*t*-test for independent data), $P < 0.01$.

Table 9. *In vitro* effects of bile acids on the binding spectra and monooxygenase activities of kidney microsomes

	Binding spectrum		Monooxygenase activity	
	Laurate (type I)	Vitamin D_3 (reverse type I)	Aminopyrine	7-Ethoxycoumarin
	(O.D. × 10^3/mg protein per ml)		(nmole/min/mg protein)	
Control	0.27 ± 0.05	2.4 ± 0.7	0.30 ± 0.07	0.007 ± 0.002
Cholate	0.27 ± 0.07	2.4 ± 0.6	0.33 ± 0.12	0.007 ± 0.002
Glycocholate	0.25 ± 0.11	3.0 ± 0.7	0.26 ± 0.07	0.005 ± 0.002
Taurocholate	0.22 ± 0.07	2.7 ± 0.8	0.32 ± 0.09	0.004 ± 0.001*
Chenodeoxycholate	0.37 ± 0.12	2.1 ± 0.6	0.37 ± 0.05	0.011 ± 0.005
Glycochenodeoxycholate	0.32 ± 0.07	2.7 ± 0.7	0.37 ± 0.03	0.001 ± 0.001*
Taurochenodeoxycholate	0.35 ± 0.17	2.3 ± 0.6	0.33 ± 0.06	0.001 ± 0.001*

Kidneys from control rats were pooled, and kidney microsomes were prepared. The amplitude of binding spectra, and monooxygenase activities were measured in the absence (control) or in the presence of various bile acids (1 mM). Results are means ± S.D. for 5 experiments. O.D., optical density.
* Significantly different from that measured in the absence of bile acid (control) (*t*-test for dependent data), $P < 0.05$.

Table 10. *In vitro* solubilizing effects of bile acids on the NADPH–cytochrome *c* reductase activity of renal microsomes

	NADPH–cytochrome *c* reductase activity	
	Microsomes	Supernatant
	(μmole/min/g kidney)	
Control	0.22 ± 0.06	0.07 ± 0.02
Cholate	0.21 ± 0.08	0.10 ± 0.03*
Glycocholate	0.19 ± 0.06	0.17 ± 0.05*
Taurocholate	0.19 ± 0.07	0.17 ± 0.05*
Chenodeoxycholate	0.20 ± 0.11	0.05 ± 0.06
Glycochenodeoxycholate	0.13 ± 0.04*	0.24 ± 0.04*
Taurochenodeoxycholate	0.16 ± 0.04*	0.20 ± 0.07*

Kidneys from control rats were pooled; renal microsomes were prepared and resuspended in buffer. Some aliquots received no addition (control). In other aliquots, various bile acids (1 mM) were added. After 60 min in ice, microsomal suspensions were centrifuged again and NADPH–cytochrome *c* reductase activity was measured both in the microsomal fraction and in the 100,000 *g* supernatant fraction. Results are means ± S.D. for 5 experiments.
* Significantly different from that measured without addition of bile acids (control) (*t*-test for independent data), $P < 0.05$.

fraction) resulted in the partial solubilization of the reductase (Table 10); cytochrome P-450 was not detectably solubilized at this concentration of bile acids (not shown).

Addition of bilirubin (1 mM) to the 10,000 *g* renal supernatant fraction did not modify the spectral measurement of cytochrome P-450 in subsequently isolated renal microsomes (not shown).

DISCUSSION

The purpose of this investigation was to determine whether a decrease in hepatic cytochrome P-450 content may increase cytochrome P-450 levels in some extrahepatic organs. To assess this hypothesis, we employed various surgical procedures (Tables 1, 4, 6) previously reported to decrease hepatic cytochrome P-450 content [30, 32–35, 38]. Sham operation itself tended to slightly decrease hepatic cytochrome P-450 at 3 or 7 days (Tables 1 and 6), but not at 35 days (not shown). Partial hepatectomy further reduced total hepatic microsomal cytochrome P-450 content, owing, first, to the reduction in liver weight, but also, to a secondary decrease in the concentration of cytochrome P-450 (Table 1), an effect possibly related to the immaturity of regenerating hepatocytes [30]. Bile duct ligation for 7 or 35 days markedly decreased hepatic cytochrome P-450 concentration (Tables 4 and 6), an effect apparently related to a reduced synthesis of cytochrome P-450 [33, 34, 47]. As a "control" situation, we also used a model of late, post necrotic cirrhosis in which there is marked liver pathology but normal hepatic cytochrome P-450 contents (Table 7) [38].

Our results show that some of these models do indeed affect cytochrome P-450 in extrahepatic organs. These extrahepatic effects were modest in lung. The microsomal cytochrome P-450 contents of the whole lungs were slightly increased in rats with biliary cirrhosis (in which renal cytochrome P-450 was most increased), but were not significantly modified in rats with partial hepatectomy or cholestasis (Tables 1, 4, 6). Extrahepatic effects were greater in the kidney. Indeed, partial hepatectomy increased renal microsomal cytochrome P-450 and tended to increase (non-significantly) NADPH–cytochrome *c* reductase activity (Fig. 1, Table 1). The 7-ethoxycoumarin deethylase activity of kidney microsomes was markedly increased while other tested monooxygenase activities or binding spectra were not significantly modified (Table 2), suggesting that partial hepatectomy may selectively increase some renal cytochrome P-450 isozyme(s). Bile duct ligation for 7 or 35 days produced related but not identical effects: these models also increased renal cytochrome P-450; however, kidney microsomal NADPH–cytochrome *c* reductase activity tended instead to decrease and the 7-ethoxycoumarin deethylase activity of kidney microsomes remained unchanged (Tables 4–6). A first possibility is that partial hepatectomy and bile duct ligation may have initially different effects on renal microsomal enzymes, increasing in particular different renal cytochrome P-450 isozymes. A second possibility, however, is that differences between the model of partial hepatectomy and those of bile duct ligation may be, in fact, explained by secondary modifications due to the interference of bile acids in the latter models. Indeed, a first effect of several bile acids *in vitro* is to partially solubilize microsomal NADPH–cytochrome *c* reductase [48]. This effect, previously reported in hepatic microsomes [48], was also observed in kidney microsomes (Table 10). Such a solubilizing effect, occurring *in vivo* in the kidneys of bile duct ligated rats, may have masked an actual induction of the reductase in these rats. Indeed, whereas the activity of NADPH–cytochrome *c* reductase tended to decrease in kidney microsomes from bile duct-ligated rats (Table 5), in contrast, this activity was markedly increased in the renal cytosol, and the total activity (microsomal + cytosolic) was significantly increased (Table 5). A second *in vitro* effect of some bile acids was to markedly inhibit the 7-ethoxycoumarin deethylase activity of kidney microsomes (Table 9); a similar inhibition of some monooxygenase activities has been previously reported in hepatic microsomes [49]. Conceivably, bile acids present *in vivo* in the kidneys of bile duct-ligated rats may persist in isolated kidney microsomes where they may inhibit the activity of 7-ethoxycoumarin deethylase. Such an inhibitory effect may have masked an actual increase in cytochrome P-450 isozymes having an intrinsically high 7-ethoxycoumarin deethylase activity.

The mechanism for the increased renal cytochrome P-450 contents observed in hepatectomized or bile duct-ligated rats cannot be definitely established from our study. A major difficulty is that the liver, in addition to being the main location of cytochrome P-450 in the body, has several other important functions: it clears several peptidic hormones released in the portal vein by pancreas and gut; it secretes in bile a number of endogenous compounds such as bilirubin, bile acids, steroids and phospholipids; it synthesizes and releases plasma proteins, lipoproteins and several other compounds. These other hepatic functions might be altered in rats with partial hepatectomy, cholestasis or biliary cirrhosis and might be responsible for the observed increase in kidney microsomal enzymes. It is noteworthy, however, that despite marked liver pathology, renal cytochrome P-450 contents remained unchanged in rats with late, postnecrotic cirrhosis. This may indicate that alterations in the above-mentioned liver functions may not be the cause for the increased renal cytochrome P-450 contents observed in the surgical models. It is also noteworthy that kidney microsomal cytochrome P-450 was increased in the 3 models in which hepatic cytochrome P-450 was decreased (partial hepatectomy, cholestasis, and biliary cirrhosis), whereas it remained unchanged in the model of late, post-necrotic cirrhosis, in which hepatic cytochrome P-450 content was unchanged. These observations are consistent with the view (see Introduction) that, in the presence of reduced hepatic cytochrome P-450 contents, some endogenous substrate(s) of cytochrome P-450 may be poorly metabolized, may attain high steady state concentrations, and may thereby induce some renal cytochrome P-450 isozyme(s). If this hypothesis is correct, it may also apply to exogenous substrates. Therefore, to indirectly assess this hypothesis, we

tested the inducing effects of an exogenous inducer, phenobarbital. Whereas this barbiturate was a poor inducer of renal cytochrome P-450 in control rats, it became a potent inducer of renal cytochrome P-450, when its metabolism was decreased by partial hepatectomy (Table 3).

We conclude (a) that renal cytochrome P-450 is increased in three models in which hepatic cytochrome P-450 is decreased (partial hepatectomy, cholestasis, and biliary cirrhosis), but remains unchanged in a model of severe liver pathology (post-necrotic cirrhosis) in which hepatic cytochrome P-450 is not decreased and (b) that phenobarbital, albeit a poor inducer of renal microsomal enzymes in control rats, becomes a potent inducer in rats with two-thirds hepatectomy. We suggest that cytochrome P-450 levels in the liver may indirectly influence the levels of some cytochrome P-450 isozyme(s) in the kidney.

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