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DRUG METABOLISM IN CIRRHOSIS

SELECTIVE CHANGES IN CYTOCHROME P-450 ISOZYMES IN THE CHOLINE-DEFICIENT RAT MODEL

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Abstract—The effect of a choline-deficient diet on microsomal cytochome P-450 and mixed-function oxidase (MFO) activity was investigated in relation to the development of nutritional cirrhosis. In rats that received the choline-deficient diet for 28 weeks cirrhosis was evident macroscopically and histologically; control rats that received an identical diet supplemented with choline had normal livers. Microsomal cytochrome P-450 and cytochrome b_5 were reduced in cirrhotic liver to 50% of control levels. Three MFO activities (ethylmorphine *N*-demethylase, aryl hydrocarbon hydroxylase and 7-ethoxycoumarin *O*-deethylase) were also reduced to 40–70% of control levels. However, the turnover number for the *O*-deethylation of 7-ethoxycoumarin was not reduced in cirrhotic liver. This finding suggested that certain drug oxidations may be selectively depressed in nutritional cirrhosis.

To examine the possibility that selective changes in MFO activity may reflect the suppression of certain cytochrome P-450 isozymes, partially purified fractions of the cytochrome were prepared after solubilisation and hydrophobic affinity chromatography (on *n*-octylamino-Sepharose 4B) of cirrhotic and control liver microsomes. Analysis of these fractions by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and laser densitometry indicated that a protein band of apparent minimum molecular weight 50.5 kD was primarily affected in cirrhotic rat liver microsomes. Levels of two other bands (apparent minimum molecular weight 48 and 52.5 kD) appeared essentially unaltered. Additional electrophoretic studies, conducted under non-reduced conditions, indicated the haemoprotein nature of protein bands in the 48–55 kD region. These data strongly suggest that cirrhosis produced in rats by a choline-deficient diet is associated with selective decreases in oxidative drug metabolism and individual cytochrome P-450 isozymes.

In patients with chronic liver disease, the elimination of drugs metabolised by the liver is often impaired. While several physiological determinants contribute to such changes [1, 2], a major underlying abnormality is decreased activity of hepatic mixed-function oxidases (MFO), the principal component of which is cytochrome P-450. It is now well established that there are multiple isozymes of cytochrome P-450 and that each isozyme is associated with a different spectrum of MFO substrate specificity. Hence the MFO activity of any liver will depend upon the relative proportion of individual isozymes present at that time.

Based upon *in vivo* studies of hepatic drug clearance [3] and *in vitro* measurement of enzyme activity [4], it has been suggested that selective alterations in different MFO activities may occur in chronic liver disease. However, although reductions in total levels of hepatic cytochrome P-450 have been observed repeatedly [4–6], there have been no direct studies of cytochrome P-450 isozyme composition in patients with chronic hepatocellular disease.

To examine the mechanisms leading to impaired drug metabolism in chronic liver disease, experimental models of cirrhosis in laboratory animals have been studied. The most commonly used model of hepatic cirrhosis is that produced by repeated exposure of rats to carbon tetrachloride (CCl_4) usually with concomitant phenobarbital administration [7, 8]. Using this model we have reported decreased levels of cytochrome P-450 with reduced capacity to bind [9] and catalyze metabolism of a range of drug substrates [10]. However, despite these reductions of cytochrome P-450 and MFO activity, total microsomal protein synthesis and the capacity for induction of cytochrome P-450 were unaffected [10] and hepatic haem turnover was normal [11]. On the basis of these observations it has been postulated that changes in cytochrome P-450 in chronic liver disease may be due to altered regulation of basal levels of the cytochrome [10]. If down-regulation of cytochrome P-450 is due to the pathophysiological state of cirrhosis and is not due to a direct effect of CCl_4 on specific forms of the cytochrome, as has been proposed to occur in acute CCl_4 toxicity [12, 13], then similar changes might be expected in other models of experimental liver disease. Moreover, because the regulation of cytochrome P-450 isozymes appears to be subject to independent control mechanisms [14, 15], it might be expected that liver disease would have a nonuniform effect on individual cytochrome P-450 isozymes.

The present study was undertaken to evaluate hepatic drug metabolising enzymes in a nutritional model of cirrhosis, the choline-deficient rat. In particular, several mixed-function oxidase activities were determined in relation to the decrease in total microsomal cytochrome P-450. Cytochrome P-450 was partially purified by hydrophobic affinity chromatography prior to electrophoretic analysis on poly-

acrylamide gels and quantitation by laser densitometry. As far as we are aware, this study includes the first documentation by direct methods of cytochrome P-450 subpopulation composition in experimental chronic liver disease.

MATERIALS AND METHODS

Chemicals. Casein and α-soyprotein for use in diets were obtained from H. E. Cottee Pty Ltd (Kirribilli, NSW) and Fielders-Gillespie (Leichhardt, NSW). Corn oil and lard were purchased through normal retail outlets. Vitamins were purchased from either Sigma Chemical Co., (St. Louis, MO) or Roche Pty Ltd, (Dee Why, NSW). Dietary salts were obtained either from Sigma Chemical Co. or Ajax Chemicals (Sydney, NSW) and were at least analytical reagent grade.

Benzo(*a*)pyrene and 7-ethoxycoumarin were purchased from Aldrich Chemical Co. (Milwaukee, WI) and ethylmorphine was from McFarlan Smith Ltd (Edinburgh).

Cholic acid (Sigma Chemical Co.) was recrystallised from ethanol prior to use in solubilisation and purification buffers. Lubrol PX and biochemicals were purchased from Sigma Chemical Co.

Chemicals for electrophoresis were obtained from Bio-rad (Richmond, CA). All other solvents and chemicals were at least AR grade.

Animals. Male Wistar rats, initial weight 90–130 g, were obtained from the Institute of Clinical Pathology and Medical Research at the Westmead Hospital and were held in individual wire cages under conditions of constant temperature (22°), humidity and lighting (12 hr light-dark cycle).

Choline-deficient diet. The choline-deficient diet was modified slightly from that described by Handler and Dubin [16] and consisted of casein 6%, α-soyprotein 6%, sucrose 58%, oils and fats 25%, cystine 0.5% and cholesterol 0.5%. Each kilogram of diet was supplemented with the following vitamins: thiamine HCl, 10 mg; riboflavin, 20 mg; pyridoxine, 10 mg; calcium pantothenoate, 100 mg; *p*-aminobenzoic acid, 100 mg; 2-methylnaphthoquinone, 5 mg; and vitamin A, 250,000 units. In addition, a salt mixture, similar to that described by Hubbell *et al.* [17], was incorporated into the choline-deficient diet in the ratio of 40.4 g/kg diet mixture. The composition of the salt mixture was as follows: $CaCO_3$ 53.5%, $MgCO_3$ 2.5%, $MgSO_4$ 3.2%, NaCl 6.8%, KCl 11%, KH_2PO_4 20.8%, $FePO_4$ $0.4H_2O$ 2.0%, KI 79 ppm, $MnSO_4$ 343 ppm, NaF 985 ppm, $CuSO_4$ 888 ppm, K_2SO_4 563 ppm and $Al_2(SO_4)_3$ 111 ppm.

Choline-supplemented diet was prepared by the addition of 0.4% choline chloride to the choline-deficient diet.

Animals received water and either the choline-deficient or supplemented diet *ad libitum* over a 28-week period up until the time of sacrifice. Blood, for serum biochemical measurements, was collected by aortic puncture prior to liver perfusion.

Preparation of microsomal fraction. Following an overnight fast rats were anaesthetised with ether and the livers perfused *in situ* with 40 ml ice-cold 0.15 M NaCl before being removed and weighed. Washed microsomal fractions were then prepared as described previously [10] except that 0.01 M potassium phosphate buffer (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA was used in place of 0.1 M potassium phosphate. The final microsomal fractions were stored as pellets frozen at −20° until required for use. All enzyme assays were performed within ten days of preparation.

Protein and enzyme assays. Microsomal protein was estimated by the method of Lowry *et al.* [18]. Cytochromes P-450 and b_5 were assayed by difference spectrometry according to standard procedures [19].

Ethylmorphine *N*-demethylase activity was assayed as previously described [20]. Aryl hydrocarbon (benzo(*a*)pyrene) hydroxylase activity was estimated from the formation of fluorescent benzo(*a*)pyrene phenols, using 3-hydroxybenzo(*a*)pyrene as the standard [4]. 7-Ethoxucoumarin *O*-deethylase activity was determined in an Aminco SPF-125 spectrofluorometer by the time-dependent formation of umbelliferone (7-hydroxycoumarin) essentially as described by Ullrich and Weber [21].

Preparation of partially purified cytochrome P-450 fractions. The partial purification of microsomal cytochrome P-450 from individual choline-deficient or control (choline-supplemented diet) rat livers was achieved by a procedure similar to that of Guengerich and Martin [22].

Individual microsomal pellets were resuspended and then solubilised by the addition of 0.1 M potassium phosphate buffer (pH 7.25) containing 20% glycerol, 0.6% sodium cholate, 0.4 mM phenylmethylsulphonyl fluoride, 1 mM 1,4-dithioerythritol and 1 mM EDTA to a final concentration of 1.5 mg protein/ml. After gentle stirring for 30 min the microsomal solution was centrifuged at 105,000 *g* for 60 min at 4°. The resultant supernatant was filtered through glass wool and then applied to an *n*-octylamino-sepharose 4B column (*ca* 1.5 × 10 cm), prepared from cyanogen bromide-activated sepharose 4B and 1,8-diaminooctane as described previously [22].

After loading, the *n*-octylamino-sepharose 4B column was extensively washed with 0.1 M potassium phosphate buffer (pH 7.25) containing 20% glycerol, 1 mM EDTA and 0.42% sodium cholate until the A_{280} of the eluate dropped to baseline levels. This procedure eluted most of the microsomal protein but no more than 5% of the applied cytochrome P-450.

The major cytochrome P-450 fraction was then eluted with glycerol/phosphate buffer that also contained 0.06% lubrol PX and 0.33% sodium cholate. Cytochrome P-450 elution from columns was followed by monitoring the A_{417} of each fraction. Fractions with $A_{417} > 0.02$ were pooled for electrophoretic analysis; the pooled fraction contained 50–70% of the applied cytochrome P-450 and no cytochrome b_5 or haemoglobin was present.

Sodium dodecylsulphate polyacrylamide gel electrophoresis. Cytochrome P-450 fractions were prepared for electrophoresis by incubation with 2% sodium dodecylsulphate and 5% 2-mercaptoethanol at 100° for 5 min. Electrophoresis was conducted in 5–15% exponential gradient gels according to the method of Laemmli [23], modified by doubling the

concentrations of Tris and glycine in all buffers and gels. This modification permitted superior resolution of individual bands in the cytochrome P-450 molecular weight region. Sample loads were 100 μl per lane and samples were electrophoresed either on the basis of equal protein (4 μg) or equal cytochrome P-450 (12 pmoles) per lane. Gels were stained for protein with Coomassie brilliant blue [24] and destained in acetic acid:methanol:water (7:5:88). Molecular weight markers (Bio-rad laboratories, Sydney) were myosin (200,000), β-galactosidase (130,000), phosphorylase B (94,000), bovine serum albumin (68,000), ovalbumin (43,000), carbonic anhydrase (29,000), soybean trypsin inhibitor (21,000) and lysozyme (14,000).

Additional electrophoretic studies were conducted using the method of Fairbanks *et al.* [24] under non-reduced conditions, except that 8% (v/v) glycerol and 3.5 M urea were included in gels. A 2 hr pre-electrophoresis step at 18 mA was introduced so that excess ammonium persulphate could be removed from gels. Partially purified cytochrome P-450 fractions (0.12 nmoles) were subjected to overnight electrophoresis at about 18 mA in the dark. Gels were subsequently stained for haemoprotein-mediated peroxidase activity with 3,3′, 5,5′-tetramethylbenzidine-hydrogen peroxide, as described by Thomas *et al.* [25].

Densitometry. Stained polyacrylamide gels were scanned with an LKB 2202 Ultroscan laser densitometer. Staining intensities of different molecular weight regions were quantified using an LKB model 2220 recording integrator.

Miscellaneous serum biochemistry. Serum biochemistry tests were performed in the Clinical Chemistry Laboratory of the Institute of Clinical Pathology and Medical Research, Westmead Hospital using automated procedures.

Analysis of data. In these experiments, mean values of data obtained from choline-deficient cirrhotic and control liver microsomes were compared using the unpaired Student's *t*-test (two-tailed).

RESULTS

The choline-deficient rat model of cirrhosis

Rats that received the choline deficient diet gained weight at an identical rate to controls that received diet supplemented with choline. Livers from choline-deficient rats were significantly greater in size (21.9 ± 2.6 g vs 10.8 ± 1.3 g, $P < 0.001$; Table 1) than control livers. Cirrhosis and fatty infiltration were apparent both by macroscopic assessment and histological examination (Fig. 1) of livers from choline deficient rats. The changes were not present in control rat livers.

Splenic weight was also significantly increased in the choline-deficient rat (1.43 ± 0.24 g vs 0.80 ± 12 g, $P < 0.001$; Table 1) probably as a result of portal hypertension. Testicular weight in choline-deficient animals was significantly lower than in choline-supplemented controls ($P < 0.02$; Table 1). However, ascitic fluid was not noted in any of the choline-deficient rats.

Table 1. Choline-deficient cirrhotic and choline-supplemented rats: body parameters

	Body weight (g)	Liver weight (g)	Microsomal protein (mg)	Microsomal protein (mg/g liver)	Testicular weight (g)	Splenic weight (g)
Choline-supplemented	370 ± 20	10.8 ± 1.3	180 ± 30	17 ± 4	2.40 ± 0.51	0.80 ± 0.12
Choline-deficient	360 ± 10	21.9 ± 2.6	140 ± 15	6.3 ± 1.0	1.69 ± 0.40	1.43 ± 0.24
	NS	$P < 0.001$	$P < 0.02$	$P < 0.001$	$P < 0.02$	$P < 0.001$

Data are mean ± S.D. of six rats (choline-supplemented) and seven rats (choline-deficient).

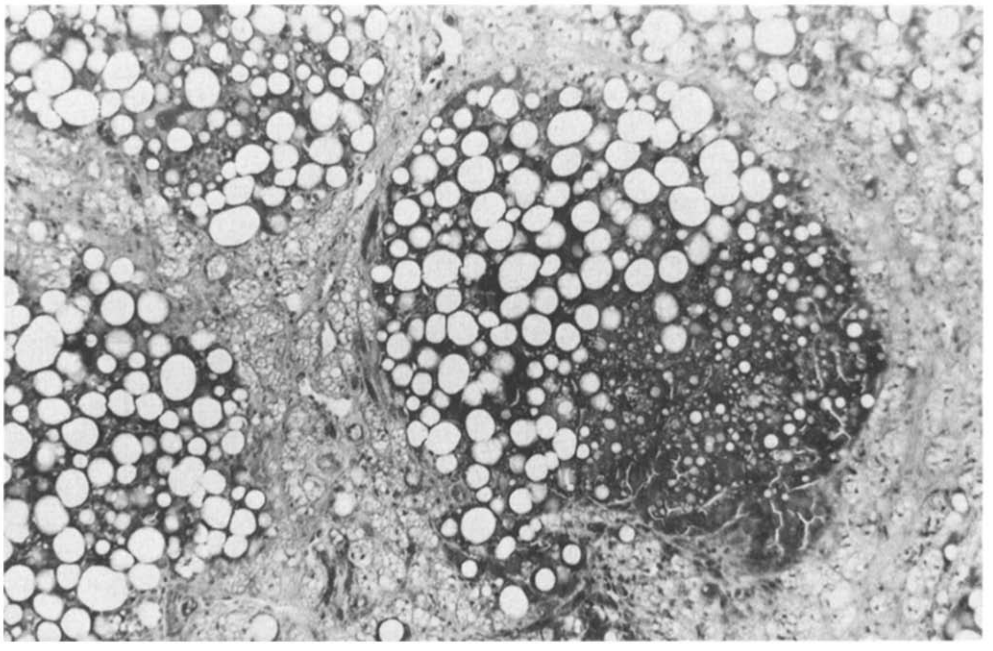

Fig. 1. Light microscopy of cirrhotic liver from a rat fed the choline-deficient diet for 28 weeks. Regeneration nodules are surrounded by fibrotic bands. Liver cells show fatty changes, and fat-laden cells are also evident in fibrous tissue septa (Mallory's trichrome, × 64).

Compared with control rats, serum levels of bilirubin, alkaline phosphatase, alanine aminotransferase, and γ-glutamyl transpeptidase were significantly increased in choline-deficient rats (Table 2). However, levels of serum albumin were not reduced in rats that received the choline-deficient diet. These data indicate that continuing hepatocellular injury and dysfunction is significant in this model of cirrhosis.

Effect of cirrhosis produced by choline-deficiency on hepatic microsomal proteins and mixed-function oxidase activities

Total hepatic microsomal protein content was significantly reduced in choline-deficient rats (6.3 ± 1.0 mg/g of wet liver vs 17 ± 4 mg/g of wet liver in controls, $P < 0.001$, Table 1). This difference is only partially a consequence of the twofold increase in hepatic mass (see above) in choline-deficient rats.

Cytochrome P-450 and cytochrome b_5 were significantly reduced in hepatic microsomes from choline-deficient rats to levels about 50% of those found in control microsomes (Table 3). As might be expected, these decreases are reflected in pronounced effects on the drug oxidation capacity of choline-deficient cirrhotic rat liver. Levels of three representative MFO activities were decreased in choline-deficient microsomes, although the extent of this reduction was nonuniform. In microsomes from choline-deficient rats, ethylmorphine *N*-demethylase and aryl hydrocarbon hydroxylase activities were reduced to about 30% of levels in controls whereas 7-ethoxycoumarin *O*-deethylase activity fell to 50% of control values (Table 4).

To evaluate further the possibility that individual MFO activities were differentially altered in the choline-deficient cirrhotic rat liver, enzyme activity was also calculated per nmole of cytochrome P-450 (Table 5). Expressed in this way, ethylmorphine *N*-demethylase and aryl hydrocarbon hydroxylase activities in microsomal fractions from choline-deficient rat liver were reduced to approximately 60 per cent of choline-supplemented controls whereas 7-ethoxycoumarin *O*-deethylase activity was not significantly reduced.

Table 2. Choline-deficient and choline-supplemented rats: serum biochemistry

	Bilirubin (μmoles/l)	Albumin (mg/ml)	Alkaline Phosphatase (units/l)	Alanine Aminotransferase (units/l)	γ-glutamyl transpeptidase (units/l)
Choline-supplemented	1.8 ± 0.4	34 ± 2	44 ± 9	26 ± 4	0.8 ± 0.4
Choline-deficient	7.3 ± 2.1	34 ± 2	155 ± 40	66 ± 19	3.0 ± 2.0
	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$	$P < 0.05$

Data are mean ± S.D. of six individual rats in each group.

Table 3. Levels of cytochromes P-450 and b_5 in liver microsomes from choline-supplemented and choline-deficient rats

	Cytochrome P-450 (nmole/mg protein)	Cytochrome b_5 (nmole/mg protein)
Choline-supplemented	1.53 ± 0.32	0.58 ± 0.09
Choline-deficient	0.73 ± 0.09	0.29 ± 0.06
Percent of choline-supplemented controls	48	50
	$P < 0.001$	$P < 0.001$

Values are mean ± S.D. of six individual rat livers in each group.

Table 4. Mixed-function oxidase activities in choline-supplemented and choline-deficient rat liver microsomes

	Ethylmorphine *N*-demethylase (nmole formaldehyde/mg protein/15 min)	7-Ethoxycoumarin *O*-deethylase (nmole umbelliferone/mg protein/min)	Aryl hydrocarbon hydroxylase (nmole 3-hydroxybenzo[*a*]pyrene/mg protein/15 min
Choline-supplemented	276 ± 26	2.03 ± 0.34	7.65 ± 0.98
Choline-deficient	85 ± 15	0.97 ± 0.29	2.13 ± 0.46
Percent of choline-supplemented controls	31	48	28
	$P < 0.001$	$P < 0.001$	$P < 0.001$

Values are mean ± S.D. of six individual rat livers in each group.

Partial purification of cytochrome P-450

The solubilisation of microsomes from both choline-deficient cirrhotic and choline-supplemented rat liver and subsequent separation by hydrophobic-affinity column chromatography on *n*-octylamino-Sepharose 4B achieved an approximate twofold increase in the specific content of cytochrome P-450. The total recovery of applied cytochrome P-450 was 80–90% (50–70% of the applied cytochrome P-450 was eluted by Lubrol PX in a single fraction).

Analysis of microsomal proteins separated by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis

The advantage of partial purification of cytochrome P-450 prior to SDS-polyacrylamide gel electrophoresis is illustrated in Fig. 2. Densitometric analysis of crude microsomal fractions is complicated by the number of bands present, even in the molecular weight range from 48 to 55 kD, which encompass the molecular weights of known forms of highly purified cytochrome P-450. Partially purified fractions contained a higher proportion of the total staining intensity (of each sample lane) within the 48–55 kD range. The haemoprotein nature of the protein bands present in this molecular weight region was confirmed by electrophoresis under non-reduced conditions. Unfortunately, as indicated by Kling *et al.* [26], these conditions do not permit adequate resolution of individual protein bands but, despite this fact, staining with both 3,3′, 5,5′-tetramethylbenzidine-H_2O_2 (haem) and Coomassie blue (protein) occurred over the same molecular weight range (results not shown).

In order to study the effects of the cirrhosis produced by choline-deficiency on relative and absolute proportions of protein bands in the cytochrome P-450 molecular weight region, fractions were subjected to electrophoresis on 5–15% gradient gels on

Table 5. Turnover numbers of mixed-function oxidase activities in choline-supplemented and choline-deficient rat liver microsomes

	Ethylmorphine *N*-demethylase (nmole product/nmole P-450/min)	7-Ethoxycoumarin *O*-deethylase (nmole product/nmole P-450/min)	Aryl hydrocarbon hydroxylase (nmole product/nmole P-450/min)
Choline-supplemented	12.4 ± 2.3	1.37 ± 0.32	0.333 ± 0.073
Choline-deficient	7.8 ± 1.0	1.15 ± 0.09	0.194 ± 0.042
Percent of choline-supplemented controls	63	84	58
	$P < 0.002$	NS	$P < 0.001$

Values are mean ± S.D. of six individual rat livers in each group.

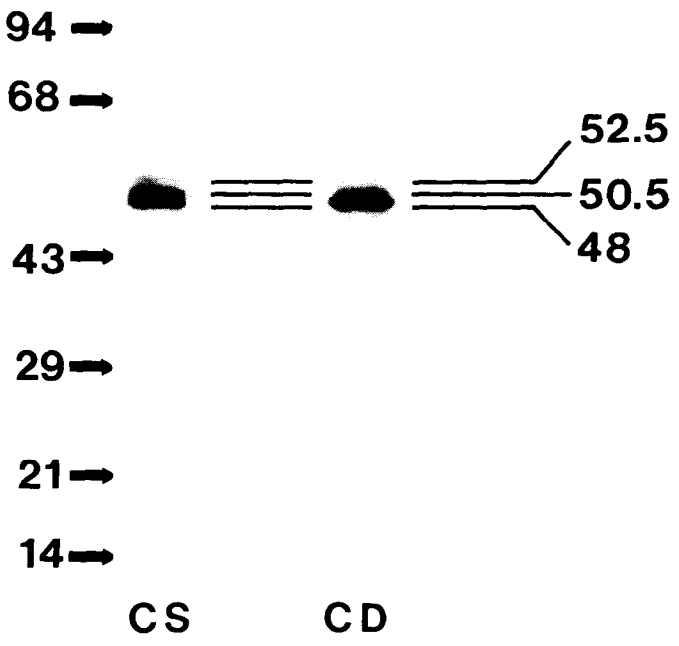

Fig. 2. An exponential 5–15% SDS-polyacrylamide gel of partially purified cytochrome P-450 fractions from choline-supplemented control (CS) and choline-deficient (CD) cirrhotic rat hepatic microsomes. The gel was run under conditions of equal cytochrome P-450 per lane and was stained for protein with Coomassie brilliant blue.

the basis of equal cytochrome P-450 as well as equal protein per lane. As shown in Table 6, three major bands were apparent in the 48–55 kD region. In fractions (equal cytochrome P-450) from control liver, the major staining band was found around 50.5 kD (52–66% of total staining intensity in the 48–55 kD range; Table 6) but in fractions from choline-deficient cirrhotic rat liver this band represented only 26–32% of the total intensity. In contrast, the major band in fractions from cirrhotic rat liver was observed at 48 kD (35–52% of total) whereas this band was less prominent in controls (18–27%). The higher molecular weight band around 52.5 kD stained as a similar percentage of total in fractions from both cirrhotic and control liver (10–23% in cirrhosis vs 13–26% in control; Table 6).

Studies in which equal amounts of protein were added to each gel lane provided additional evidence for selective effects on cytochrome P-450 isoenzymes in cirrhotic liver (Fig. 3; Table 6). Whereas the staining in both 48 and 52.5 kD bands appeared equivalent in cirrhotic and control fractions, the staining intensity of the 50.5 kD band in cirrhotic rat liver was only about one-third that of controls.

Table 6. Laser densitometric analysis of protein bands from partially purified cytochrome P-450 fractions on polyacrylamide gels

	Percentage of staining intensity in each molecular weight band		
	48,000	50,500	52,500
Equal cytochrome P-450 per lane			
Choline-supplemented	18–27	52–66	13–26
Choline-deficient	35–52	26–32	10–23
Equal protein per lane			
Choline-supplemented	18–24	56–65	11–24
Choline-deficient	19–36	20–23	10–16

Values are ranges of percent staining intensities of each molecular weight region (N = 3).

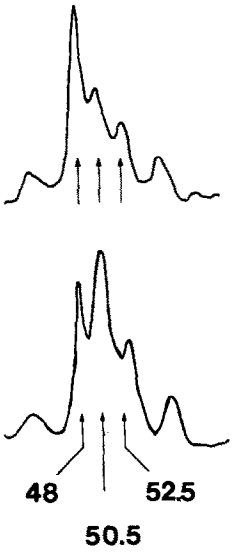

Fig. 3. Laser densitometric profile of stained proteins on 5–15% SDS-polyacrylamide gels in the cytochrome P-450 molecular weight region. Protein profiles of partially purified hepatic cytochrome P-450 fractions from choline-deficient cirrhotic (upper trace) and choline-supplemented (lower trace) rats are shown. Profiles were obtained after densitometry of stained gels run under conditions of equal protein per lane.

DISCUSSION

The prolonged intake of a diet which is deficient in choline but rich in lipid produces cirrhosis in rats [16, 27] and has some features in common with alcoholic cirrhosis in humans [28]. The present study establishes that cirrhosis produced by chronic intake of a choline-deficient diet is associated with a 50% decrease in levels of microsomal cytochrome P-450 and decreased mixed-function oxidase activity compared with choline-supplemented control liver. However, the reduction in MFO activity was disproportionately low compared with total cytochrome P-450.

The present study provides convincing evidence that cirrhosis has a selective effect on individual hepatic MFO activities. When catalytic activity was expressed in terms of product formed per nmol of cytochrome P-450 (turnover number), cirrhosis was not associated with a reduction of microsomal 7-ethoxycoumarin *O*-deethylase activity. However, a significant decrease in ethylmorphine *N*-demethylase and aryl hydrocarbon hydroxylase activities to approximately 60% of control was apparent. Earlier *in vitro* studies of hepatic MFO activity in patients with liver disease have also suggested that effects on some enzymes are more pronounced than on others [4]. These changes would be expected to result in variable effects on the intrinsic hepatic clearance of particular drugs in patients with severe liver disease. Such observations have been made following *in vivo* studies [1, 3] although translation of these changes into effects on drug elimination rates depends also on other physiological determinants such as hepatic blood flow and binding to plasma proteins [2].

The present data strongly suggest that the most likely explanation for selective alterations in MFO activity is that individual cytochrome P-450 isozymes are differentially affected in cirrhosis produced by choline deficiency. Following partial purification of cytochrome P-450 fractions by hydrophobic affinity chromatography, cytochrome P-450 isozymes were resolved into three major fractions on SDS-polyacrylamide gels. When the relative staining intensity of each of these bands were compared by laser densitometry, it was clearly evident that the relative proportions of each band were different in choline-deficient cirrhotic rats compared with controls.

It is now well established that at least six to nine cytochrome P-450 isozymes are present in hepatic microsomes of noninduced rats, although the levels of many are extremely low [29, 30]. The techniques used in the present study do not resolve all cytochrome P-450 isozymes, and it is therefore probable that each gel band contains a number of proteins. Nevertheless, the partial purification step of hydrophobic affinity chromatography results in a major improvement in resolution of cytochrome P-450 isozyme subpopulations by SDS-polyacrylamide gel electrophoresis.

Another possible explanation for the disproportionate reduction of MFO activity relates to the well-documented changes in liver phospholipid, specifically decreased phosphatidylcholine, which is known to occur after intake of a choline-deficient diet [31–34]. Studies with reconstituted enzyme systems have shown that optimal cytochrome P-450-dependent MFO activity requires phosphatidylcholine [35], probably to facilitate the information of an active cytochrome P-450: NADPH-cytochrome P-450 reductase complex [36] as well as for optimal substrate binding [37]. It follows that, in the choline-deficient model of cirrhosis, the defect in phosphatidylcholine level may impair the assembly of MFO components within the hepatic endoplasmic reticulum. It is also possible that alterations in hepatic phospholipid may selectively decrease certain MFO activities. A recent study by Ioannoni *et al.* [38] has demonstrated the importance of phospholipid in the observation of a high affinity binding interaction between the H_2-receptor antagonist cimetidine and a semi-purified cytochrome P-450 preparation from untreated rat liver. In contrast, phospholipid was not essential for the binding reaction between cimetidine and phenobarbitone-induced rat cytochrome P-450.

In earlier studies, we examined cytochrome P-450 function and turnover in rats with cirrhosis produced by repeated CCl_4 administration. A limitation of this approach is that changes attributed to cirrhosis may have been caused instead by the toxic agent (CCl_4). This remains a potential problem even though the final exposure of rats to CCl_4 is ten days before experiments are conducted. In view of this factor it was considered important to compare changes in microsomal function in a different model of cirrhosis. The changes in cytochrome P-450 levels and function found in the choline-deficient cirrhotic rat liver resemble those in cirrhosis produced by repeated CCl_4 administration. Certainly, dietary choline-deficiency and CCl_4 both produced impairment of the hepatic endoplasmic reticulum, but it appears

likely that at least some of the effects observed in these models may be attributed to active cirrhosis and may therefore carry relevance for the defects in hepatic drug metabolism found in human liver disease.

The present data provide the most convincing evidence to date that chronic liver disease is associated with selective depression of certain cytochrome P-450 isozymes. It seems very likely that this selective reduction of particular isozymes is responsible for alterations in the binding and metabolism of different drug substrates by the liver. Outstanding questions concern whether constitutive and/or inducible forms of cytochrome P-450 are altered in cirrhosis and whether changes are due to altered regulation of particular isozymes or to selective damage of individual proteins. Immunoquantitation of cytochrome P-450 isozymes, and applications of these techniques to other models is now essential to define more precisely the selective effects of cirrhosis on cytochrome P-450 isozyme composition and turnover.

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