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HEPATIC DRUG METABOLISM IN RATS WITH EXPERIMENTAL CHRONIC RENAL FAILURE*

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Abstract—The activities of several hepatic microsomal, mitochondrial, and cytosolic drug-metabolizing enzymes, as well as the components of the cytochrome P-450 system, were determined *in vitro* for control, sham-operated, and uremic rats. Chronic renal failure (CRF) was produced by a two-stage surgical procedure. In this model, the animals were maintained for 21 days postoperatively before assay. During this time, serum urea nitrogen (SUN) levels rose from control levels of 21 mg/dl to an average of 63 mg/dl. Enzymes assayed included microsomal *N*-, *O*-, and *S*-demethylases, esterase, and UDP-glucuronyl transferase; monoamine oxidase; and alcohol dehydrogenase. CRF caused decreases of 24–32% in *N*- and *O*-demethylase activities, while *S*-demethylase, esterase, UDP-glucuronyl transferase, and monoamine oxidase activities were not altered significantly. Alcohol dehydrogenase activity was increased 71%. In addition, the functional components of the microsomal mixed-function oxidase system were assayed. CRF caused a 26% decrease in cytochrome P-450 levels, as compared to sham-operated controls, but cytochrome b_5 and NADPH-cytochrome *c* (P-450) reductase were not altered. CRF caused an increase in hexobarbital sleeping time of more than 7-fold. In each case, alterations in enzyme activities or cytochrome P-450 correlated with the extent of renal failure, as determined by elevated SUN levels.

Malfunction of the kidneys results in an array of symptoms which reflect pathological alterations in every organ system of the body [1]. As a consequence of the medical problems which result from the disease, chronic renal failure patients are treated frequently with several different classes of pharmacological agents. Despite the adjustment of dosage to compensate for reduced renal clearance, the frequency and severity of adverse drug reactions are significantly greater in patients with renal failure than in patients who do not suffer from renal insufficiency [2, 3]. Clinical studies have shown that the metabolism of certain drugs may be altered by renal failure [4–6]. These alterations may contribute to the observed increase in toxicity of drugs in these patients.

Investigations of experimental acute [7–9] and chronic [10] renal failure in animals have also shown alterations in certain pathways of drug metabolism. No systematic study of the effects of chronic renal failure on drug-metabolizing activity in all hepatic cell fractions has yet been performed. Therefore, we have determined the *in vitro* activities of a wide variety of hepatic drug-metabolizing enzymes in rats with a surgically produced model of chronic renal failure. The activities investigated are present in microsomal, mitochondrial, and cytosolic fractions of the liver, including the major components of the microsomal cytochrome P-450 system [i.e. cytochrome P-450, NADPH–cytochrome *c* (P-450) reductase, and cytochrome b_5].

MATERIALS AND METHODS

Chemicals. [2-^{14}]Tryptamine bisuccinate, 55.2 mCi/mmole, was obtained from the New England Nuclear Corp. (Boston, MA); purity was verified by thin-layer chromatography. *o*-Aminophenyl-β-D-glucuronide, cytochrome *c* (Type III), and enzyme cofactors were the highest purity obtainable from the Sigma Chemical Co. (St. Louis, MO). Aminopyrine, reagent grade, was obtained from the Aldrich Chemical Co. (Milwaukee, WI); codeine phosphate, U.S.P. and ethylmorphine HCl, N.F. from Merck & Co. (Rahway, NJ); hexobarbital sodium (Evipal), U.S.P. from Winthrop Laboratories (New York, NY); and phenobarbital sodium, U.S.P. from Mallinckrodt & Co. (St. Louis, MO). All other chemicals used were reagent grade and obtained from commercial sources.

Surgical procedure. Male Sprague–Dawley rats (Sprague–Dawley Laboratories, Madison, WI), 40- to 60-days-old, weighing approximately 200 g, were housed four to six per cage and fed a commercial diet (Wayne Laboratory Chow) *ad lib*. Partial nephrectomy was produced surgically in two stages under ether anesthesia by a modification of the procedure originally developed by Parrish and Oller [11] as described by Hayslett *et al.* [12]. Stage one consisted of laparotomy and ligation of the upper and lower

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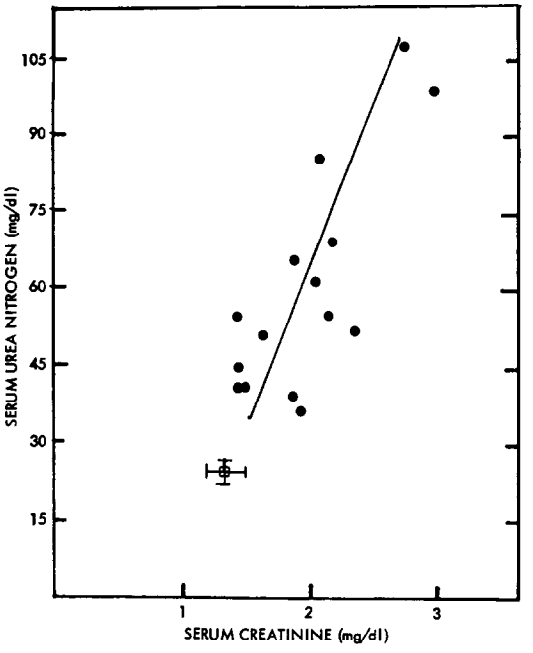

Fig. 1. Correlation of serum urea nitrogen (SUN) with serum creatinine values. CRF rats (N = 15) were killed 21 days postoperatively. SUN and serum creatinine were determined in triplicate for each animal. Each point represents the mean of each determination in one rat. Values for the control group (N = 7) are shown as one point which represents the mean ± S.E. for each parameter. $r^2 = 0.74$ ($P < 0.05$).

poles of the left kidney to occlude 50–70% of the renal mass. The animals were allowed to recover for 7 days. Stage two consisted of laparotomy and right nephrectomy. The adrenal glands were left intact. The procedure is estimated to eliminate 70–85% of the functional renal mass. Sham-operated (SHAM) animals were subjected to laparotomy and kidney decapsulation on the same days as the chronic renal failure (CRF) group. Control (CONT) animals were untreated.

The extent of renal failure was determined in all experiments by the elevation of serum urea nitrogen (SUN). Twenty days after the second stage of surgery, 1-ml samples of tail vein blood were collected after snipping off the tip of the tail. SUN was determined by the method of Crocker [13]. To determine their validity as a measure of renal function, SUN values were correlated with serum creatinine values in a group of fifteen CRF rats and seven CONT rats (Fig. 1). Serum creatinine was determined using a kit (Hycel, Inc., Houston, TX). This correlation was necessary because the production of urea by the liver may increase during experimental acute renal failure in rats [14–16]. The data (Fig. 1) indicate that SUN alterations are a valid index of the extent of renal failure in this model.

Tissue preparation. Twenty-one days after the second stage of surgery, rats were killed by cervical dislocation. Rats were killed in groups of four animals per day which included two CRF rats, one SHAM rat, and one CONT rat. Livers were excised, rinsed in ice-cold 1.15% (w/v) KCl in 0.05 M sodium-potassium phosphate buffer, pH 7.4, weighed, and homogenized in a Tissumizer (Tekmar Co., Cincinnati, OH) in 4 vol. of the 1.15% KCl-buffer. A portion of the crude homogenate was centrifuged for 20 min (Beckman J21-B, Beckman Instruments Inc., Palo Alto, CA) at 2–5°. The 10,000 *g* supernatant fraction was used as enzyme source in most experiments. A portion was further centrifuged at 100,000 *g* for 60 min (Beckman L3-50 centrifuge) at 2–5°. The 100,000 *g* supernatant fraction was separated, and the microsomal pellet was resuspended in the same buffer used for homogenization.

Enzyme assays. Unless otherwise specified, enzyme activities were determined by aerobic incubation at 37° in a Dubnoff-type incubator. Preliminary experiments established concentrations of substrate and cofactor necessary to ensure zero-order kinetics and linearity with respect to incubation time and enzyme concentration. With the exception of UDP-glucuronyl transferase (see below), all assays were performed on the same day, using freshly prepared hepatic cell fractions.

Microsomal assays. The demethylations of aminopyrine, ethylmorphine, codeine, and 6-methylmercaptopurine riboside (6-MMPR) were determined by assaying the production of formaldehyde using the Nash procedure [17] as modified by Cochin and Axelrod [18]. Incubation mixtures consisted of 3 μmoles $NADP^+$; 30 μmoles glucose-6-phosphate; 15 μmoles $MgCl_2$; 7.5 μmoles semicarbazide; 225 μmoles sodium-potassium phosphate buffer, pH 7.4; 100 mg rat liver as the 10,000 *g* supernatant fraction; and either aminopyrine (75 μmoles), ethylmorphine HCl (45 μmoles), codeine phosphate (45 μmoles), or 6-MMPR (15 μmoles) in a final volume of 3.0 ml. Reactions with aminopyrine were incubated for 20 min; reactions with ethylmorphine, codeine, or 6-MMPR were incubated for 30 min.

β-Naphthyl acetate esterase activity was determined by assaying the formation of β-naphthol using the method of Nachlas and Seligman [19] as modified by Imai *et al.* [20]. Incubation mixtures consisted of 15 μg rat liver as a diluted 10,000 *g* supernatant fraction; 30 μmoles barbital buffer, pH 7.4; and 12.5 μmoles β-naphthyl acetate in a final volume of 1.0 ml. Reactions were incubated for 20 min.

UDP-glucuronyl transferase activity was determined by assaying the formation of *o*-aminophenyl-β-D-glucuronide from UDP-glucuronic acid and *o*-aminophenol using a modification of the method of Dutton and Storey [21]. UDP-glucuronyl transferase activity is activated *in vitro* by storage, detergents, and certain nucleotides [22, 23]. Therefore, to obtain maximal activation of enzyme activity, assays were performed on microsomal suspensions that had been stored for 4 days at 5°. Incubation mixtures consisted of 40 mg rat liver as a microsomal suspension; 100 μmoles Tris–HCl buffer, pH 7.4; 30 μmoles $MgCl_2$; 10 μmoles UDP-glucuronic acid; and 210 μmoles *o*-aminophenol in a final volume of 2.0 ml. Reactions were incubated for 10 min.

NADPH–cytochrome *c* reductase (NADPH–cytochrome P-450 reductase) activity was determined by following the formation of the reduced form of cytochrome *c* using the method of Williams and

Kamin [24] as described by Mazel [25]. Enzyme activity was determined by measuring the initial rate of formation of reduced cytochrome *c* by changes in absorbance at 550 nm in a Beckman Acta CIII recording spectrophotometer at 25°.

Cytochrome P-450 was assayed by the method of Omura and Sato [26] as described by Mazel [25].

Cytochrome b_5 was assayed by the method of Strittmatter and Velick [27] as described by Mazel [25].

Mitochondrial enzyme assay. Monoamine oxidase activity was determined by assaying the formation of [^{14}C]indole acetate from [^{14}C]tryptamine using the method of Wurtman and Axelrod [28]. Radioactivity was determined using a Beckman DPM-100 liquid scintillation spectrometer. Counting efficiency was 93%.

Cytosolic enzyme assay. Alcohol dehydrogenase was assayed by following the formation of NADH from NAD^+ and ethanol using a modification of the method of Bonnichsen and Brink [29]. Incubation mixtures consisted of 100 μmoles glycine–NaOH buffer, pH 10.5; 8 mg NAD^+; 20 mg of rat liver as 100,000 *g* supernatant fraction; and 0.015% (v/v) ethanol in a final volume of 3.0 ml. Enzyme activity was determined by following the initial rate of formation of NADH at 340 nm in a recording spectrophotometer at 25°.

Protein assay. Protein content of hepatic cell fractions was determined by the method of Lowry *et al.* [30] using bovine serum albumin as a standard.

Hexobarbital sleeping time. Sodium hexobarbital (150 mg/kg, i.p.) was administered to a group of CRF and control rats 1 day before they were killed for *in vitro* assays to determine the effect of CRF upon *in vivo* drug metabolism. Sleeping time was defined as the duration of loss of the righting reflex. The experiment was performed in a quiet room with no external stimuli.

Induction of hepatic drug metabolism with phenobarbital. Four CRF rats and seven SHAM rats were administered phenobarbital sodium (40 mg/kg, i.p.) once per day for 4 days, starting 17 days postoperatively. The animals were then killed, and *in vitro* assays were performed as above.

Data analysis. Statistically significant differences in data were determined using Student's *t*-test or one-way analysis of variance and the Newman–Keuls test as appropriate. Differences were judged to be statistically significant when the probability of α error was less than 5% ($P < 0.05$).

RESULTS

In vitro hepatic drug-metabolizing activities were determined in twenty-eight CRF, twelve SHAM, and thirteen CONT rats. The data are shown in Table 1. Aminopyrine, ethylmorphine, codeine, and 6-MMPR were used as model substrates for *N*-, *O*-, and *S*-demethylation pathways of hepatic biotransformation. Table 1 shows that the microsomal *N*- and *O*-demethylase activities were statistically significantly decreased by CRF, but *S*-demethylase remained unaltered. The average decreases in activity were 24% for aminopyrine *N*-demethylase, 32% for ethylmorphine *N*-demethylase, and 31% for codeine *O*-demethylase. Sham surgery caused no significant change in any of the demethylase activities. Although there was an apparent increase (33%) in microsomal β-naphthyl acetate esterase activity and a decrease (19%) in UDP-glucuronyl transferase activity, neither of these reached statistical significance. There was a significant decrease (26%) in the amount of cytochrome P-450 in the CRF group as compared to

Table 1. Alterations in hepatic drug metabolism produced by chronic renal failure*

	CONT	SHAM	CRF	r^2
Aminopyrine *N*-demethylase	2.72 ± 0.18	2.87 ± 0.14	2.06 ± 0.11†‡	0.15§
Ethylmorphine *N*-demethylase	2.50 ± 0.10	2.71 ± 0.17	1.71 ± 0.17†‡	0.51§
Codeine *O*-demethylase	2.40 ± 0.23	2.50 ± 0.17	1.65 ± 0.10†‡	0.28§
6-Methylmercaptopurine riboside *S*-demethylase	0.65 ± 0.23	0.76 ± 0.07	0.61 ± 0.03	0.14
β-Naphthyl acetate esterase	394 ± 52	570 ± 60	525 ± 39	0.09
UDP-glucuronyl transferase	0.73 ± 0.05	0.65 ± 0.04	0.59 ± 0.04	0.06
NADPH–cytochrome *c* (P-450) reductase	109 ± 3	120 ± 6	101 ± 5	0.04
Cytochrome P-450	0.61 ± 0.05	0.74 ± 0.03†	0.55 ± 0.03‡	0.19§
Cytochrome b_5	0.57 ± 0.04	0.55 ± 0.02	0.54 ± 0.02	0.18§
Monoamine oxidase	0.64 ± 0.02	0.66 ± 0.02	0.62 ± 0.01	0.03
Alcohol dehydrogenase	13.9 ± 1.0	12.4 ± 1.6	23.7 ± 1.8†‡	0.18§
Animal weight	298 ± 6	295 ± 5	254 ± 8‡	
Liver weight	11.8 ± 0.4	11.8 ± 0.4	10.0 ± 0.5‡	
Microsomal protein	29 ± 1.0		29 ± 1.0	
Serum urea nitrogen (SUN)	21 ± 1	21 ± 1	63 ± 6†‡	
Animals/group	13	12	28	

* Enzyme activities are expressed as nmoles product/mg protein/min. Cytochromes are expressed as nmoles/mg protein. Animal and liver weights are g/animal and microsomal protein is mg/g liver. SUN values are mg/dl. Data are given as mean ± S.E. for all animals in that group.

† $P < 0.05$ compared to control group.

‡ $P < 0.05$ compared to sham group.

§ $P < 0.05$ for correlation of activity with SUN in CRF animals.

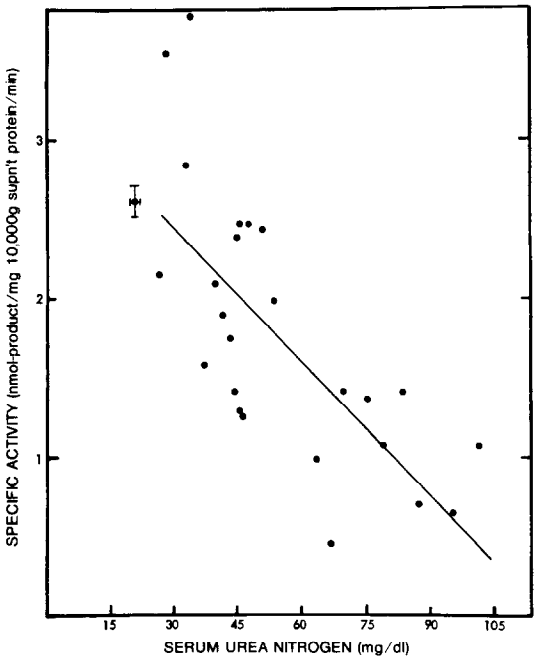

Fig. 2. Inverse correlation of ethylmorphine *N*-demethylase activity with serum urea nitrogen (SUN) values. Same animals as Table 1. Each point represents mean values for one rat from triplicate determinations. Values for control and sham-operated rats are shown as one point which represents the mean ± S.E. for each parameter. The regression line is based only on data from CRF rats. $r^2 = 0.51$ ($P < 0.05$).

the SHAM group. Sham surgery caused a statistically significant increase (21%) in cytochrome P-450 as compared to controls. The other components of the cytochrome P-450 mixed-function oxidase system, NADPH–cytochrome *c* (P-450) reductase and cytochrome b_5, were not altered significantly by CRF, however.

The effects of CRF upon hepatic biotransformation were not restricted to microsomal activities. The cytosolic enzyme alcohol dehydrogenase was statistically significantly elevated (71%) by CRF as compared to controls. The mitochondrial enzyme monoamine oxidase was not altered significantly by CRF.

Average SUN values were elevated significantly for the CRF group (63 ± 6 mg/dl) as compared to either the SHAM (21 ± 1 mg/dl) or CONT (21 ± 1 mg/dl) groups. The extent of alteration of each significantly altered enzyme activity and of cytochrome P-450 levels could be correlated with increased SUN values. Figure 2, for example, shows the inverse relationship between ethylmorphine *N*-demethylase activity and SUN. The coefficients of determination (r^2) for each enzyme activity and for each component of the cytochrome P-450 mixed-function oxidase system are included in Table 1.

Hexobarbital sleeping time was determined in another group of five CRF rats and four control rats as an index of *in vivo* hepatic microsomal drug-metabolizing activity. CRF caused a 7-fold increase in sleeping time, from 12 ± 3.5 min in the control group to 89 ± 19 min in the CRF group. Although the *in vitro* metabolic data for these animals are not presented here, the results obtained in each group were similar to the results presented in Table 1.

It was of interest to determine whether the loss of mixed function oxidase (MFO) activity and the decrease in P-450 levels could be reversed by the inductive effects of phenobarbital. Table 2 shows that *N*- and *O*-demethylase activities, NADPH–cytochrome *c* (P-450) reductase, cytochrome P-450, and microsomal protein could all be increased significantly by phenobarbital pretreatment of CRF rats. Phenobarbital pretreatment did not, however, alter microsomal β-naphthyl acetate esterase activity or that of alcohol dehydrogenase. The percent increases in microsomal *N*- and *O*-demethylases, NADPH–cytochrome *c* (P-450) reductase, and cytochrome P-450 after phenobarbital pretreatment were greater for the CRF group (column four) than for the sham-operated controls (column three).

DISCUSSION

The present investigation has examined the effect of experimental chronic renal failure (CRF) on a broad spectrum of hepatic drug-metabolizing pathways. Microsomal, mitochondrial, and cytosolic enzyme activities were determined, as well as the components of the cytochrome P-450 mixed function oxidase (MFO) system. Each of the microsomal oxidative enzyme activities was decreased by CRF; however, only the *N*- and *O*-demethylases and cytochrome P-450 were decreased to the level of statistical significance. Several other microsomal enzyme activities and components of the P-450 MFO system were not altered significantly by CRF, however. These included 6-MMPR *S*-demethylase, UDP-glucuronyl transferase, β-naphthyl acetate esterase, NADPH–cytochrome *c* (P-450) reductase, and cytochrome b_5. Hexobarbital sleeping time, an *in vivo* index of microsomal MFO activity, was increased significantly in these animals. The mitochondrial enzyme monoamine oxidase was also not affected by CRF, but the cytosolic enzyme alcohol dehydrogenase was increased significantly by CRF.

Thus, the changes brought about by CRF on microsomal enzymes are not generalized but show some apparent specificity. The basis for this selective sensitivity is not known, but it is currently being investigated.

Other investigators have also shown changes in certain drug-metabolizing activities using models of acute or chronic renal failure. Leber and Schutterle [7] showed that aminopyrine *N*-demethylase, *p*-nitroanisole *O*-demethylase, acetanilide hydroxylase, and cytochrome P-450 were decreased in their rat model of acute (6 days) renal failure (ARF); cytochrome b_5 remained unaltered, however. Mezey *et al.* [8] showed that alcohol dehydrogenase activity was increased; cytochrome P-450 was decreased; and aminopyrine *N*-demethylase, aniline hydroxylase, cytochrome *c* reductase, and cytochrome b_5 remained unaltered by ARF (10 days). In each of these studies, ARF was induced in rats by surgical subtotal nephrectomy. Van Peer and Belpaire [9] produced ARF in rabbits with uranyl nitrate and reported that aminopyrine *N*-demethylase was decreased while aniline

Table 2. Effect of phenobarbital (PB) pretreatment on *in vitro* drug metabolism of chronic renal failure and sham-operated control rats‡

	PB-pretreated		Relative activity†	
	SHAM	CRF	SHAM	CRF
Aminopyrine *N*-demethylase	5.75 ± 0.36‡	4.79 ± 0.56‡	200	233
Ethylmorphine *N*-demethylase	7.67 ± 0.38‡	4.94 ± 0.62‡	283	289
Codeine *O*-demethylase	5.16 ± 0.38‡	3.95 ± 0.39‡	206	239
β-Naphthyl acetate esterase	588 ± 41	460 ± 83	103	88
NADPH–cytochrome *c* (P-450) reductase	302 ± 38‡	324 ± 62‡	252	321
Cytochrome P-450	1.91 ± 0.07‡	1.63 ± 0.15‡	258	296
Cytochrome b_5	0.55 ± 0.02	0.52 ± 0.06	100	96
Microsomal protein	40.0 ± 0.7‡	36.0 ± 1.5‡	138	124
Alcohol dehydrogenase	21.8 ± 1.6	33.6 ± 1.4	118	113
Serum urea nitrogen (SUN)	30 ± 3	91 ± 19		

* Rats in the chronic renal failure (CRF) group (N = 4) and sham-operated controls (SHAM) (N = 7) were administered phenobarbital sodium (40 mg/kg, i.p.) daily for 4 days, starting 17 days postoperatively, and then killed. Aminopyrine, ethylmorphine, and codeine demethylases, β-naphthyl acetate esterase, SUN, and protein were determined in triplicate. NADPH–cytochrome *c* (P-450) reductase and alcohol dehydrogenase were determined in duplicate. Cytochrome P-450 and cytochrome b_5 were single determinations. Enzyme activities are expressed as nmoles product/mg protein/min. Cytochromes are expressed as nmoles/mg protein. Microsomal protein is expressed as mg/g liver and SUN as mg/dl. Data are given as mean ± S.E. for all animals in that group.

† Non-pretreated = 100 (Table 1).

‡ $P < 0.05$ compared to corresponding non-pretreated group (Table 1).

hydroxylase remained unaltered at both day 2 and 6 post-treatment.

Leber *et al.* [10] also used a surgically produced model of CRF (45 days) in rats to show that aminopyrine *N*-demethylase, *p*-nitroanisole *O*-demethylase, acetanilide hydroxylase, and cytochrome P-450 were decreased by CRF. Related to this was an increase in hexobarbital sleeping time. Hydrolysis of acetanilide and *p*-nitrophenylacetate, reduction of prontosil, and glucuronidation of *p*-nitrophenol and 4-methylumbelliferone remained unaltered by CRF.

One consequence of the surgical procedure used to induce CRF was a significant loss of body and liver weights, although the liver weight per 100 g body weight remained unaltered (3.96 for CONT and 3.94 for CHR rats). Since sham-operated animals did not lose body weight, the weight loss of the CRF group may be attributed to the anorexic effects of CRF. CRF did not alter the protein content of liver cell fractions in this model. The use of specific enzyme activities, recorded in Table 1, reflects intrinsic alterations of drug-metabolizing activity irrespective of changes in liver weight. Starvation of rats has been shown to cause specific alterations in hepatic MFO activities. For example, Kato and Gillette [31] reported a decrease in aminopyrine *N*-demethylase and hexobarbital oxidase activities after rats were starved for 48 hr. It is unlikely, however, that the changes we observed in CRF can be attributed to anorexia and its attendant changes in drug metabolism. Leber and Schutterle [7] utilized a calorically deprived group as control for their ARF and showed no alterations in cytochrome P-450, aminopyrine *N*-demethylase, or acetanilide hydroxylase; *O*-demethylation of *p*-nitroanisole was increased. Further, Bode *et al.* [32] demonstrated that fasting caused a decrease in alcohol dehydrogenase activity in rats. Since partial caloric deprivation produces opposite effects on alcohol dehydrogenase activity compared to CRF, it is unlikely that anorexia caused the metabolic alterations noted in the present investigation.

Despite the standardized surgical procedure, CRF rats exhibited considerable variation in their extent of renal impairment, as measured by SUN on postoperative day 20. This large range in values could be due to variability in the amount of viable renal mass which remained after surgery. A statistically significant correlation was found between the enzyme activities and SUN values. Figure 2, for example, shows that 51% of the change in ethylmorphine *N*-demethylase activity can be related to changes in SUN values. Changes in other microsomal, mitochondrial, and cytosolic enzymes are shown in Table 1, along with their correlation with SUN.

Although the alterations in enzyme activity are correlated with SUN values, increased amounts of urea *per se* are not likely to be the cause of the activity changes. Mezey *et al.* [8] showed that addition of urea or uremic plasma *in vitro* had no effect on liver alcohol dehydrogenase activity in rats. Further, Ivanetich *et al.* [33] showed that addition of amines to rat liver microsomal preparations does not affect the components of the cytochrome P-450 MFO system. At best, the coefficients of determination in Table 1 suggest that only 18–51% of the variation in enzyme activity can be related to changes in SUN.

The administration of phenobarbital to a group of CRF and control rats showed that the inductive effects on microsomal MFO activities and components of the P-450 system were not compromised by CRF. In fact, the components and activities appeared to be more inducible in the CRF group, as reflected by the greater percent increases in the CRF group after phenobarbital administration (Table 2). Although phenobar-

bital induction of microsomal drug metabolism in uremic animals has not been reported previously, Leber *et al.* [10] showed a slight increase of microsomal drug-metabolizing activities after daily treatment of uremic rats with di(2-ethylhexyl)phthalate (DEHP) at a dose of 200 mg/kg for 14 days. DEHP is a plasticizer which is known to leach from polyvinyl dialysis tubing into the bloodstream and affect some liver enzyme activities [34, 35]. In addition, rats with experimental acute uremia are known to respond to well known inducing agents [7, 36].

Previous investigators have shown that alterations in the content of cytochrome P-450 and *in vitro* microsomal MFO activities can be correlated with altered *in vivo* drug responses [37–40]. This would imply that, in renal failure, drugs whose activation or inactivation is dependent upon *N*- or *O*-demethylation or alcohol dehydrogenase may have altered pharmacokinetics beyond that attributable to alterations in renal clearance. This will be particularly true if the altered enzyme activity represents the rate-limiting step in the metabolism of the drug. In contrast, presumably because the rate of *in vivo* ethanol metabolism is limited by the availability of NAD^+ rather than alcohol dehydrogenase (ADH), previous studies have shown a lack of correlation between the *in vitro* activity of ADH and *in vivo* rates of ethanol elimination [41–45].

This investigation and the other related studies suggest that physicians should be made aware that, in patients with renal insufficiency, alterations in the response to a pharmacological agent may occur not only as the result of its decreased renal clearance but also as an effect of an altered ability of the liver to metabolize it to active or inactive products.

REFERENCES

1. J. P. Knochel and D. W. Seldin, in *The Kidney* (Eds. B. M. Brenner and F. C. Rector), p. 1448. W. B. Saunders, Philadelphia (1976).
2. C. J. McAllister, E. B. Scowden and W. J. Stone, *Clin. Nephrol.* **10**, 191 (1978).
3. J. W. Smith, L. G. Seidl and L. E. Cluff, *Ann. intern. Med.* **65**, 629 (1966).
4. S. Scherrer, B. Haldimann, A. Kupfer, F. Reubi and J. Bircher, *Clin. Sci. molec. Med.* **54**, 133 (1978).
5. M. M. Reidenberg, D. T. Lowenthal, W. Briggs and M. Gasparo, *Clin. Pharmac. Ther.* **20**, 67 (1976).
6. R. Verbeeck, T. B. Tjandramaga, R. Verberckmoes and P. J. DeSchepper, *Br. J. clin. Pharmac.* **3**, 1033 (1976).
7. H. W. Leber and G. Schutterle, *Kidney Int.* **2**, 152 (1972).
8. E. Mezey, R. E. Vestal, J. J. Potter and J. W. Rowe, *J. Lab. Clin. Med.* **86**, 931 (1975).
9. A. P. Van Peer and F. M. Belpaire, *Archs int. Pharmacodyn. Ther.* **228**, 180 (1977).
10. H. W. Leber, L. Gleumes and G. Schutterle, *Kidney Int.* (Suppl.) **8**, S-43 (1978).
11. A. Parrish and D. Oller, *Clin. Res.* **16**, 392 (1968).
12. J. P. Hayslett, M. Kashgarian and F. H. Epstein, *J. clin. Invest.* **48**, 1002 (1969).
13. C. L. Crocker, *Am. J. med. Technol.* **33**, 361 (1967).
14. E. C. Persike and T. Addis, *Am. J. Physiol.* **158**, 149 (1949).
15. A. L. Sellers, J. Katz and J. Marmorston, *Am. J. Physiol.* **191**, 345 (1957).
16. W. W. Lacy, *Am. J. Physiol.* **216**, 1300 (1969).
17. T. Nash, *Biochem. J.* **55**, 416 (1953).
18. J. Cochin and J. Axelrod, *J. Pharmac. exp. Ther.* **125**, 105 (1959).
19. M. M. Nachlas and A. M. Seligman, *J. biol. Chem.* **181**, 343 (1949).
20. Y. Imai, A. Ito and R. Sato, *J. Biochem., Tokyo* **60**, 417 (1966).
21. G. J. Dutton and I. D. E. Storey, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 5, p. 159. Academic Press, New York (1962).
22. K. K. Lueders and E. L. Kuff, *Archs Biochem. Biophys.* **120**, 198 (1967).
23. B. M. Pogell and L. F. Leloir, *J. biol. Chem.* **236**, 293 (1961).
24. C. H. Williams, Jr. and H. Kamin, *J. biol. Chem.* **237**, 587 (1962).
25. P. Mazel, in *Fundamentals of Drug Metabolism and Drug Disposition* (Eds. B. N. LaDu, H. G. Mandel and E. L. Way), p. 573. Williams & Wilkins, Baltimore (1971).
26. T. Omura and R. Sato, *J. biol. Chem.* **293**, 2370 (1964).
27. P. Strittmatter and S. F. Velick, *J. biol. Chem.* **221**, 253 (1956).
28. R. J. Wurtman and J. Axelrod, *Biochem. Pharmac.* **12**, 1439 (1963).
29. R. K. Bonnichsen and N. G. Brink, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 1, p. 495. Academic Press, New York (1955).
30. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
31. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150**, 279 (1965).
32. C. H. Bode, H. Goebell and M. Stahler, *Z. ges. exp. med.* **152**, 111 (1970).
33. K. M. Ivanetich, S. Lucas, J. A. Marsh, M. R. Ziman, I. D. Katz and J. J. Bradshaw, *Drug Metab. Dispos.* **6**, 218 (1978).
34. K. Ono, R. Tabukawa and T. Wakimota, *J. Am. med. Ass.* **234**, 948 (1975).
35. J. K. Reddy, D. E. Moody, D. L. Azarnoff and M. S. Rao, *Life Sci.* **18**, 941 (1976).
36. H. W. Leber, P. Streitzig, M. Kayser and G. Schutterle, *Verh. dt. Ges. Nephrol.* **8**, 75 (1971).
37. S. A. Cucinell, A. H. Conney, M. Sansur and J. J. Burns, *Clin. Pharmac. Ther.* **6**, 420 (1965).
38. M. Corn and J. F. Rockett, *Med. Ann. Distr. Columbia* **34**, 578 (1965).
39. J. R. Mitchell, H. J. Zimmerman, K. G. Ishak, U. P. Thorgeirrson, J. A. Timbrell, W. R. Snodgrass and S. D. Nelson, *Ann. intern. Med.* **84**, 181 (1976).
40. E. S. Vesell and G. T. Passanti, *Drug Metab. Dispos.* **1**, 402 (1973).
41. J. A. Edwards and D. A. P. Evans, *Clin. Pharmac. Ther.* **8**, 824 (1967).
42. E. Mezey and F. Tobon, *Gastroenterology* **61**, 707 (1971).
43. K. Zuppinger, J. Papenberg and P. Scurch, *Schweiz. med. Wschr.* **97**, 1110 (1967).
44. G. L. S. Pawan, *Nature, Lond.* **220**, 374 (1968).
45. H. Theorell and B. Chance, *Acta chem. scand.* **5**, 1127 (1951).