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Effect of Unilateral Nephrectomy on *in Vitro* Hepatic Microsomal Oxidative Drug Metabolism in the Rat (40867)[1]

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Abstract. The effect of ablation of renal mass on *in vitro* hepatic microsomal oxidative drug metabolism was studied in unilaterally nephrectomized rats which were not uremic. When compared to either sham-operated or unoperated control rats, hepatic microsomal cytochrome P-450 content and ethylmorphine *N*-demethylase activity were both decreased significantly at 4 days but not at 15 days after unilateral nephrectomy. Differences were not observed in hepatic microsomal cytochrome b_5 content or NADPH-cytochrome *c* reductase activity at either 4 or 15 days following unilateral nephrectomy. These results demonstrate that the removal of renal mass can influence temporarily *in vitro* hepatic microsomal oxidative drug metabolism and that this effect is apparent in the absence of uremia.

The interrelationship between renal function and hepatic microsomal oxidative drug metabolism has been investigated primarily in uremic conditions. Although a consistent pattern of altered hepatic microsomal oxidative drug metabolism has not been observed in patients with uremia (1–3), decreases in hepatic microsomal cytochrome P-450 content and oxidative drug metabolism have been observed in animals made uremic by removal of 60 to 85% of the total renal mass (4–6). To investigate further the effect of ablation of renal mass on the ability of the liver to oxidatively metabolize drugs, *in vitro* hepatic microsomal oxidative drug metabolism was examined after rats had undergone unilateral nephrectomy, a process in which removal of renal mass is not associated with uremia.

Materials and methods. Chemicals. Horse heart cytochrome *c* (Type VI), NADPH (Type III), and NADH (Grade III) were obtained from Sigma Chemical Company (St. Louis, Mo.). Ethylmorphine hydrochloride was purchased from Merck and Company, Inc. (Rahway, N.J.). All other biochemicals and chemicals used were of the highest purity available.

Animals. Male Holtzman rats weighing 120–140 g were used in this study. Rats which underwent surgery were anesthetized with sodium pentobarbital (35 mg/kg, ip). One group of rats was subjected to unilateral nephrectomy, while a second group underwent a sham-operation which consisted of exposure and manual manipulation of one kidney. *In vitro* hepatic microsomal oxidative drug metabolism was then studied both 4 and 15 days after surgery.

Preparation of hepatic microsomes. Unoperated control rats, sham-operated rats, and unilaterally nephrectomized rats were fasted for 24 hr and were sacrificed by decapitation. Blood was collected from unilaterally nephrectomized and sham-operated rats for measurement of serum urea nitrogen and creatinine concentrations. Livers were excised after perfusion *in situ* with ice-cold 0.154 *M* NaCl, and 10% (w/v) homogenates were prepared in ice-cold 0.25 *M* sucrose. The hepatic microsomal fraction was prepared as described by Masters *et al.* (7). Protein was determined by the biuret method using bovine serum albumin as the standard (8).

Analytical procedures. The contents of cytochromes P-450 and b_5 in hepatic microsomal suspensions were determined from

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carbon monoxide and NADH-reduced minus oxidized difference spectra, respectively, as described by Omura and Sato (9, 10). Hepatic microsomal NADPH-cytochrome *c* reductase activity was determined at 25° as described by Masters *et al.* (7). The *N*-demethylation of ethylmorphine catalyzed by hepatic microsomes was determined at 25° by measuring the rate of formation of formaldehyde employing the method of Nash (11) as modified by Cochin and Axelrod (12). Each 7-ml reaction mixture contained 14 mg of microsomal protein, 8 m*M* ethylmorphine, 150 m*M* KCl, 10 m*M* $MgCl_2$, and 50 m*M* Tris–HCl buffer, pH 7.4. After the addition of 200 μM NADPH, 1-ml aliquots were removed every 30 sec for the determination of formaldehyde.

Serum urea nitrogen and creatinine concentrations were determined using standard autoanalyzer procedures.

Results. The effect of unilateral nephrectomy on *in vitro* hepatic microsomal oxidative drug metabolism was studied initially 4 days after surgery. As seen in Table I, significant differences were not observed among the unilaterally nephrectomized, sham-operated control, and unoperated control groups with respect to body weight, liver wet weight, yield of hepatic microsomal protein, hepatic microsomal cytochrome b_5 content, or hepatic microsomal NADPH–cytochrome *c* reductase activity. However, the content of hepatic microsomal cytochrome P-450 and the hepatic microsomal ethylmorphine *N*-demethylase activity were both significantly decreased 4 days after unilateral nephrectomy (Table I). The data presented in Table I further show that significant differences in hepatic microsomal cytochrome P-450 content and in ethylmorphine *N*-demethylase activity were not observed between sham-operated and unoperated control rats, thereby demonstrating that the sham operation does not affect hepatic microsomal oxidative drug metabolism.

To investigate the long-term effect of unilateral nephrectomy on hepatic microsomal drug oxidase activity, studies were also conducted 15 days after surgery. As seen in Table II, significant differences were not observed among the three groups of rats with respect to body weight, liver wet weight, yield of hepatic microsomal protein, hepatic microsomal cytochrome b_5 content, or hepatic microsomal NADPH–cytochrome *c* reductase activity. In contrast to the results observed 4 days after

TABLE I. EFFECT OF UNILATERAL NEPHRECTOMY ON BODY WEIGHT, LIVER WET WEIGHT, AND HEPATIC MICROSOMAL OXIDATIVE DRUG METABOLISM 4 DAYS AFTER SURGERY[a]

Parameter	Unilateral nephrectomy	Sham-operated control	Unoperated control
Body weight (g)	132 ± 7	139 ± 7	142 ± 7
Liver wet weight (g)	5.8 ± 0.3	6.4 ± 0.2	6.0 ± 0.2
Microsomal protein (mg/g liver)	9.2 ± 0.4	8.4 ± 0.5	8.7 ± 0.3
Cytochrome P-450 (nmole/mg protein)	0.69 ± 0.02[b]	0.81 ± 0.02	0.85 ± 0.03
Ethylmorphine *N*-demethylase activity (nmole HCHO formed/min/mg protein)	3.1 ± 0.2[b]	4.1 ± 0.3	4.4 ± 0.2
Cytochrome b_5 (n = 5) (nmole/mg protein)	0.34 ± 0.01	0.34 ± 0.05	0.36 ± 0.02
NADPH–cytochrome *c* reductase activity (n = 5) (nmole cytochrome *c* reduced/min/mg protein)	101 ± 4	100 ± 8	107 ± 7

[a] Data given represent the mean ± SEM, n = 7 except where specifically noted. Data were analyzed using a one-way analysis of variance. If significant variation was found, Tukey's test was performed to determine significant group differences (13).

[b] $P < 0.05$ when compared to either the sham-operated control or the unoperated control group.

TABLE II. EFFECT OF UNILATERAL NEPHRECTOMY ON BODY WEIGHT, LIVER WET WEIGHT, AND HEPATIC MICROSOMAL OXIDATIVE DRUG METABOLISM 15 DAYS AFTER SURGERY[a]

Parameter	Unilateral nephrectomy	Sham-operated control	Unoperated control
Body weight (g)	246 ± 10	252 ± 12	253 ± 14
Liver wet weight (g)	9.5 ± 0.6	9.8 ± 0.5	10.1 ± 0.6
Microsomal protein (mg/g liver)	9.9 ± 0.6	10.6 ± 0.7	10.9 ± 0.8
Cytochrome P-450 (nmole/mg protein)	0.81 ± 0.03	0.84 ± 0.04	0.81 ± 0.03
Ethylmorphine *N*-demethylase activity (nmole HCHO formed/min/mg protein)	5.6 ± 0.3	6.0 ± 0.3	5.7 ± 0.4
Cytochrome b_5 (nmole/mg protein)	0.35 ± 0.01	0.35 ± 0.01	0.34 ± 0.01
NADPH–cytochrome *c* reductase activity (nmole cytochrome *c* reduced/min/mg protein)	109 ± 5	107 ± 4	105 ± 5

[a] Data given represent the mean ± SEM, $n = 8$, and were analyzed using a one-way analysis of variance.

unilateral nephrectomy, significant decreases in the content of hepatic microsomal cytochrome P-450 and in hepatic microsomal ethylmorphone *N*-demethylase activity were not observed 15 days after unilateral nephrectomy (Table II).

Renal function in unilaterally nephrectomized rats was ascertained by determining serum urea nitrogen and creatinine concentrations. Both at 4 and at 15 days after surgery, the serum urea nitrogen concentration was slightly, but significantly, greater in unilaterally nephrectomized rats than in sham-operated controls (Table III). However, it must be noted that the serum urea nitrogen concentration in the unilaterally nephrectomized rats remained below the level which is considered to be indicative of uremia (6). Significant differences were not observed in the serum creatinine concentration at either 4 or 15 days after surgery.

Discussion. The findings reported in this communication demonstrate that significant decreases in both hepatic microsomal cytochrome P-450 content and ethylmorphine *N*-demethylase activity occur 4 days after rats have undergone unilateral nephrectomy. Although the differences observed in hepatic microsomal cytochrome P-450 content and ethylmorphine *N*-demethylase activity between unilaterally nephrectomized and sham-operated control rats are relatively small (15 and 24%, respectively), these results do demonstrate that removal of renal mass can influence *in*

TABLE III. SERUM UREA NITROGEN AND CREATININE CONCENTRATIONS IN UNILATERALLY NEPHRECTOMIZED AND SHAM-OPERATED CONTROL RATS[a]

	Serum urea nitrogen (mg/100 ml)	Creatinine (mg/100 ml)
Four days after surgery ($n = 7$)		
Unilateral nephrectomy	23.3 ± 0.5[b]	1.3 ± 0.1
Sham-operated control	17.5 ± 0.8	1.1 ± 0.1
Fifteen days after surgery ($n = 6$)		
Unilateral nephrectomy	20.2 ± 0.9[b]	0.92 ± 0.03
Sham-operated control	15.3 ± 0.8	0.87 ± 0.04

[a] Data given represent the mean ± SEM and were analyzed using Student's *t* test.
[b] $P < 0.05$ when compared to the sham-operated group.

vitro hepatic microsomal oxidative drug metabolism and that this effect is observed in the absence of uremia: the mean serum urea nitrogen concentration in unilaterally nephrectomized rats 4 days after surgery was 23 mg/100 ml, or only 33% greater than in sham-operated rats. Although decreases of similar or only slightly greater magnitudes in both hepatic microsomal cytochrome P-450 content and hepatic microsomal oxidative drug metabolism have been observed in animals which have been subjected to subtotal nephrectomy (4–6), a procedure in which 60 to 85% of the total renal mass is removed, the serum urea nitrogen concentration in these animals ranged from 72 to 370 mg/100 ml (4–6), with 45 mg/100 ml being considered as indicative of a uremic condition (6). While it is possible that the decrease in hepatic microsomal ethylmorphine *N*-demethylase activity observed 4 days after unilateral nephrectomy resulted directly from the slight, but significant, elevation in serum urea nitrogen concentration, the serum urea nitrogen concentration remains significantly elevated in unilaterally nephrectomized rats for at least 15 days after surgery, with no significant difference observed in *in vitro* hepatic microsomal drug oxidase activity in these animals at that time.

The decrease in hepatic microsomal oxidative drug metabolism observed 4 days after unilateral nephrectomy may be a result of compensatory renal hypertrophy, the process in which the remaining kidney increases both in size and functional capacity. This possibility exists since it is well established that hepatic microsomal oxidative drug metabolism is influenced by the endocrinological state of the organism (14), and since endocrine as well as renotropic factors have been suggested to be responsible for compensatory renal hypertrophy (15, 16). Compensatory growth of the remaining kidney in rats, as determined by measurements of kidney weight, is fairly rapid during the week following unilateral nephrectomy, and most of the compensatory growth has occurred by day 15 (16, 17). Therefore, if the factors involved in compensatory renal hypertrophy are responsible for the observed decrease in *in vitro* hepatic microsomal drug oxidase activity, it is understandable why differences are observed in hepatic microsomal ethylmorphine *N*-demethylase activity 4 days, but not 15 days, after unilateral nephrectomy. This explanation is consistent with the recent observation by Mimnaugh *et al.* (18) that *in vitro* drug metabolism in the contralateral lung is altered during the period of compensatory lung growth following unilateral pneumonectomy. It is also consistent with the fact that decreases in *in vitro* hepatic microsomal oxidative drug metabolism are observed only during the regeneration period following partial hepatectomy (19).

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