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Characterization of a Hepatic Congestion Model in the Rat: Application to Pharmacological Studies (41035)

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Abstract. Models for producing toxic liver injury and cirrhosis have been developed in rats and applied to the study of drug pharmacokinetics, but lack of a well-characterized model of hepatic congestion has prevented previous investigation of the effects of elevated hepatic venous pressure on drug metabolism in the rat. Chronic hepatic congestion was produced in rats by partial ligation of the inferior vena cava immediately below the diaphragm and above the entrance of the hepatic veins. Significant elevation of hepatic venous pressure, prolongation of prothrombin time, and pericentral fibrosis, and sinusoidal dilitation on histological examination were seen 6 weeks after initial surgery. Liver weight in the hepatic congestion group was significantly increased when compared to sham-operated controls, while no significant difference was seen between the two groups with regard to body weight gain, bile flow, bile acid output, serum bilirubin, and alanine aminotransferase. Pentobarbital pharmacokinetic parameters were determined after intravenous administration of [^{14}C]pentobarbital to animals with hepatic congestion and sham-operated control animals. Plasma disappearance of pentobarbital was prolonged in rats with hepatic congestion and systemic plasma clearance was reduced. Additional studies of drug metabolism in this rat model of hepatic congestion may suggest important questions for future clinical investigation in man.

Effects of toxic liver injury and cirrhosis on drug pharmacokinetics have been assessed in animals (1–4), but there has been little previous study of the effects of hepatic congestion on hepatic drug disposition. Hepatic congestion occurs commonly in medical conditions such as heart failure, and additional data on the influence of hepatic congestion on hepatic drug disposition and drug pharmacokinetics are needed. While methods for producing toxic hepatic injury and cirrhosis in the rat have been refined (5, 6), there has not been validation and characterization of a model for hepatic congestion in the rat. The purpose of this paper is to describe a method for producing hepatic congestion in the rat which is accompanied by significant biochemical, physiological, and histological alterations, and to present preliminary results of application of this model to the study of drug pharmacokinetics.

Materials and Methods. Male Sprague–Dawley rats weighing 205–225 g were lightly anesthetized with ether. After exposure of the liver through a horizontal hypochondrial incision, the liver was gently retracted and a 4-O silk suture was placed around the inferior vena cava immediately below the diaphragm and above the entrance of the hepatic veins. A metal rod (1.2 mm diameter) was included within the ligature to prevent complete obliteration of the vena cava in experimental animals, and the rod was withdrawn once the suture was tied. The suture was left untied in the sham-operated control animals. After recovery from anesthesia, animals were caged and allowed standard Purina rat chow and water *ad libitum* until subsequent study.

Hepatic vein pressures were measured immediately after placement of the ligature and at 2 and 48 hr and 6 weeks postoperatively in experimental and sham animals. A manometer constructed of a 23-gauge needle and a length of Silastic medical tubing (0.5 mm i.d.) were used to measure venous pressures. After filling the tubing with isotonic saline colored with dilute indocyanine green, the needle was inserted into a hepatic vein proximal to the ligature and the height of the liquid meniscus in

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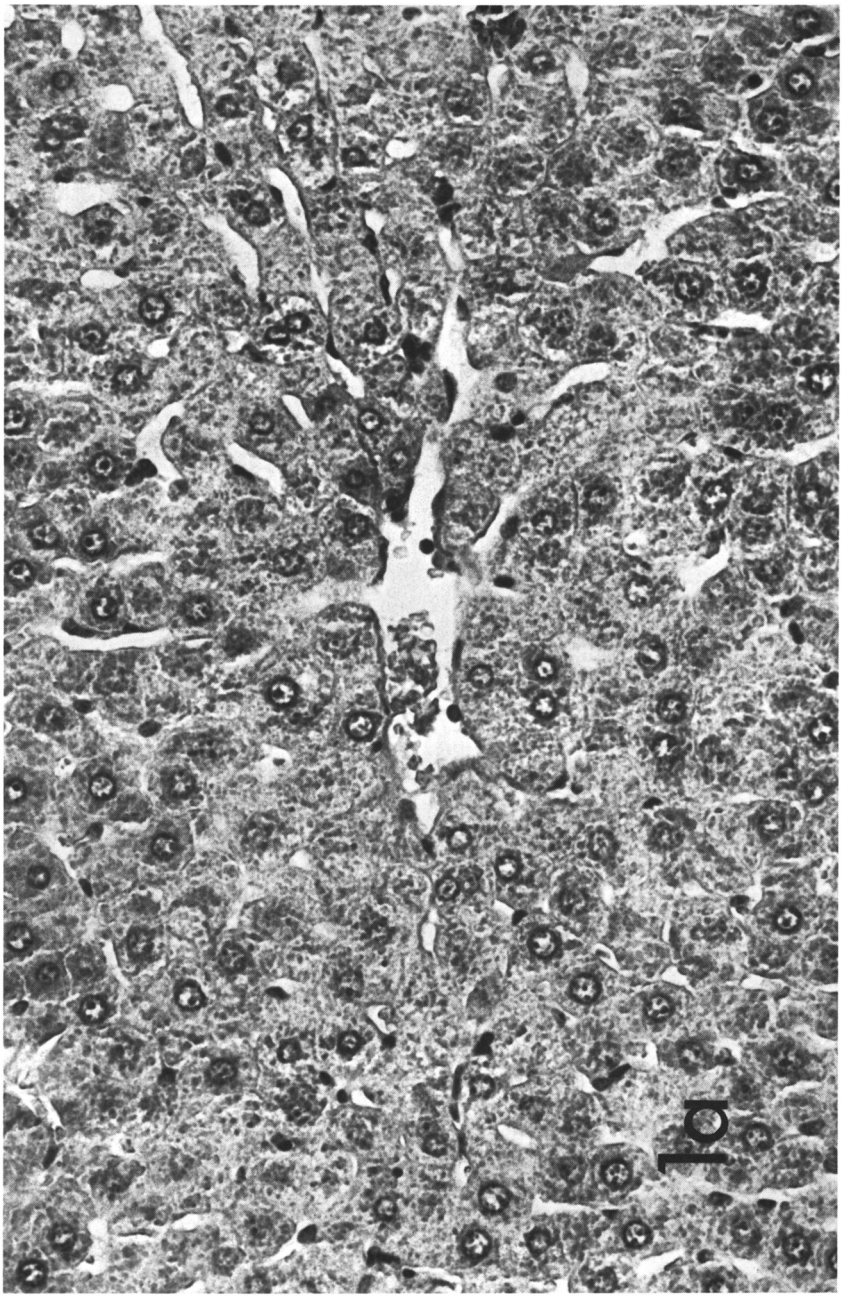
centimeters was recorded as hepatic venous pressure. Good respiratory fluctuation in meniscus height was seen and the mean of three readings of hepatic venous pressure in each animal was used. Hepatic vein pressure was measured on only one occasion in each animal.

Liver tissue was obtained for histological examination in experimental and control animals immediately after vena cava ligation or sham surgery and subsequently at laparotomy 48 hr and 6 weeks following initial surgery. Slides were stained with the periodic acid Schiff–hematoxylin, phloxine, saffron stain (7), and the Masson–Trichrome stain and examined under code. Blood was obtained by aortic puncture for biochemical testing. Prothrombin times were manually determined by the method of Quick (8), and the person performing the determination did not know from which treatment group the blood specimen had been obtained. Serum alanine aminotransferase (SGPT) levels were determined spectrophotometrically with reagents obtained from Sigma Chemical Company, St. Louis, Missouri. Total serum bilirubin was measured using an SMA 660 multichannel biochemical autoanalyzer (Technicon, Tarrytown, N.Y.).

Results of initial studies indicated that consistently elevated hepatic vein pressures, histological abnormalities, and biochemical alterations were seen at the 6-week examination following partial vena cava ligation. Therefore, subsequent characterization of the model was performed using animals who had had experimental ligation or sham surgery 6 weeks prior to further study. Under ether anesthesia the bile duct was catheterized with PE-10 polyethylene tubing (Clay Adams, Parsippany, N.J.) and the animals were placed in restraint cages. After a 2-hr period to recover from anesthesia, bile was collected in tarred vials at 30-min to 1-hr intervals for up to 4 hr. Bile flow was determined gravimetrically and bile acid concentrations were measured by the hydroxysteroid dehydrogenase method (9). Body temperatures were monitored using a Tele-Thermometer (Yellow Springs Instrument Company, Yellow Springs, Ohio) and maintained at 37 ± 1° with heating pads.

Preliminary studies of the effect of hepatic congestion on *in vivo* pentobarbital pharmacokinetics have been performed. [^{14}C]Ethyl-5-(1-methylbutyl) barbituric acid (pentobarbital) was obtained from New England Nuclear, Boston, Massachusetts, and mixed with unlabeled pentobarbital (Abbott Laboratories, North Chicago, Ill.) to give a concentration of 25 mg/ml and a specific activity of 0.6 μCi/mg. The femoral artery and vein were catheterized with PE-50 polyethylene tubing and animals were allowed to recover for 2 hr in restraint cages. [^{14}C]Pentobarbital 40 mg/kg, was then administered into a femoral vein and 0.2 ml of arterial blood was obtained at 3, 6, 9, 15, 30, 45, 60, 90, 120, 150, and 180 min for analysis of plasma pentobarbital concentrations. Unmetabolized pentobarbital was extracted from plasma using the method of Kuntzman (10), and radioactivity in the extract was determined by liquid scintillation counting. Area under the plasma concentration versus time curve ($AUC_{0-\infty}$) was calculated by the trapezoidal rule for observed values and then extrapolated to infinity (11), and other pentobarbital pharmacokinetic parameters were determined using the $AUC_{0-\infty}$ data according to standard formulas (12). Unpaired *t* analysis was used to determine significant differences between the sham-operated and hepatic congestion groups (13).

Results. The mortality rate in the immediate and early postoperative period (≤24 hr) was 38% for the partial vena cava ligation group and negligible for the sham-operative procedure. Livers of experimental animals were grossly congested and discolored immediately after and 2 hr postligation, and hepatic venous pressures measured 15 ± 1 cm water as compared to 5 ± 1 cm water in the sham-operated group. However, at 48 hr postoperatively hepatic venous pressure had fallen to 8 ± 2 cm water and no consistent morphological changes of hepatic congestion could be identified on light microscopy examination of liver tissue. Six weeks following partial vena cava ligation, hepatic venous pres-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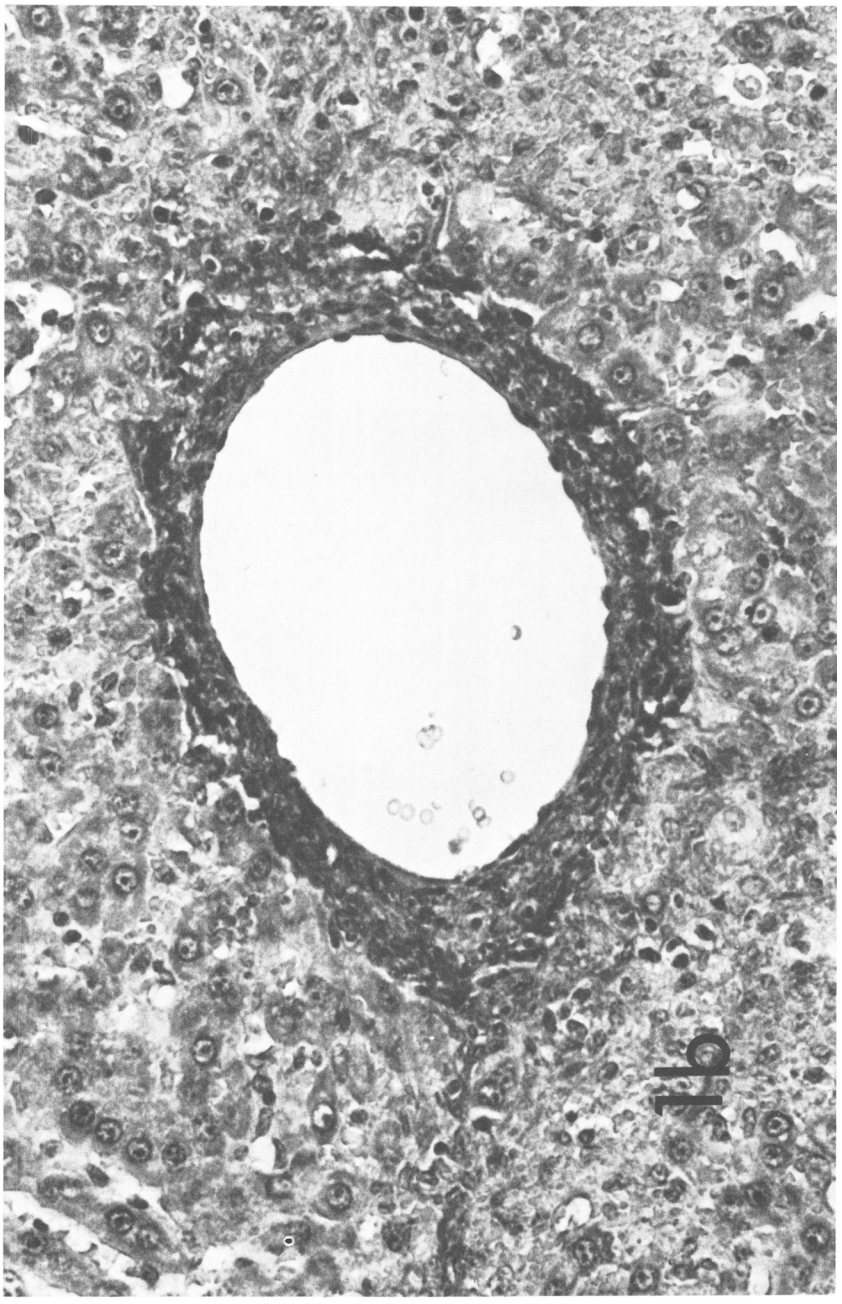

FIG. 1. Central vein and surrounding hepatic parenchyma from a liver of a typical sham-operated control animal (a) and an animal wth hepatic venous congestion induced by partial vena cava ligation (b). Masson–Trichrome stain, magnification 40×.

sures were significantly elevated in experimental animals as compared to sham-operated controls (15 ± 1 vs 9 ± 2 cm H_2O, $P < 0.025$), and histological changes of hepatic congestion including central vein dilatation and marked pericentral fibrosis were readily apparent (Fig. 1). No gross ascites was evident in the peritoneal cavity in any experimental animal.

Additional data characterizing this rat model of hepatic congestion were obtained in animals 6 weeks postsurgery and are listed in Table I. No significant difference in weight gain was seen between the two groups. No alteration in bile flow or bile acid output expressed per gram of liver weight was seen, but liver weight expressed as percentage of body weight was significantly increased in the hepatic congestion group ($P < 0.005$). Biochemically, no significant difference between the two groups was seen for serum total bilirubin or alanine aminotransferase, but prothrombin times were significantly prolonged for animals with hepatic congestion ($P < 0.001$).

Composite pentobarbital plasma disappearance curves are shown for sham-operated controls and experimental animals with hepatic congestion in Fig. 2. Pharmacokinetic parameters calculated from plasma disappearance data are given in Table II. Delayed elimination of pentobarbital from plasma occurred in animals with hepatic congestion.

Discussion. Yates originally described a surgical procedure of partial inferior vena caval ligation to produce hepatic congestion in conjunction with studies on sodium and potassium balance and mineralocorticoid metabolism (14). However, parameters of hepatic physiology such as bile flow and bile acid output were not assessed in this study, and no biochemical tests indicative of hepatic injury or dysfunction were per-

TABLE I. PHYSICAL AND BIOCHEMICAL CHARACTERISTICS OF RAT HEPATIC CONGESTION MODEL[a]

	Sham-operated group[b]	Hepatic congestion group[b]	Statistical significance[c]
Weight gain[d] (% of initial body wt)	65 ± 7	61 ± 6	NS
Liver weight[d] (% of final body wt)	2.77 ± 0.09	3.31 ± 0.10	$P < 0.005$
Bile flow[d] (μl/min/g liver)	1.89 ± 0.13	1.79 ± 0.06	NS
Bile acid output[d] (nmole/min/g liver)	27 ± 3	24 ± 2	NS
Hepatic venous pressure[e] (cm water)	9 ± 2	15 ± 1	$P < 0.025$
Prothrombin time[e] (sec)	8 ± 1	20 ± 2	$P < 0.001$
Serum alanine aminotransferase[e,f] (units/ml)	34 ± 5	40 ± 10	NS
Total serum bilirubin[g] (mg/dl)	0.12 ± 0.04	0.17 ± 0.05	NS

[a] Mean values ± SEM.
[b] Values for animals studied 6 weeks after initial vena cava ligation or sham surgery.
[c] Not significant.
[d] Sham-operated, $n = 11$; hepatic congestion, $n = 12$.
[e] $n = 4$ for both study groups.
[f] Normal ≤35 Wroblenski–LaDue units/ml.
[g] Sham-operated, $n = 8$; hepatic congestion, $n = 6$.

TABLE II. *IN VIVO* PENTOBARBITAL PHARMACOKINETIC PARAMETERS FOR SHAM-OPERATED AND HEPATIC CONGESTION RATS[a]

	β (min^{-1})	$T½_{\beta}$ (min)	Systemic plasma clearance (ml/min/100 g body wt)
Sham-operated (n = 4)	0.0084 ± 0.0008	85 ± 8	1.06 ± 0.18
Hepatic congestion (n = 4)	0.0050 ± 0.0005	141 ± 11	0.81 ± 0.10

[a] Mean values ± SEM.

formed. More importantly, no morphological changes suggesting chronic hepatic congestion were seen on histological examination of liver tissue. It is not clear from this initial paper when liver histology was examined, and differences in surgical technique, in the interval between surgery and histological examination, or in the strain of rat used may explain the discordance between the observations of Yates and those of this study. It is clear that a period of time greater than 48 hr (6 weeks was used in these studies) must be allowed postoperatively for effects of hepatic congestion on hepatic biochemical parameters and histology to be evident.

Application of this model to study of the effects of hepatic congestion on *in vitro* hepatic drug metabolism and *in vivo* drug pharmacokinetics is currently underway. Data from Fig. 2 and Table II suggest that chronic hepatic congestion may produce significant alterations in hepatic drug metabolism. It will be difficult to apply pharmacological and pharmacokinetic data accumulated in this animal model of hepatic congestion to man. However, it is interesting to note that prothrombin time prolongation, the most pronounced biochemical abnormality seen in this rat model of hepatic congestion, has also been reported to be the most sensitive indicator of hepatic dysfunction in humans with congestive heart failure (15). Results of pharmacological studies using this animal model may suggest important questions for future clinical investigation in man.

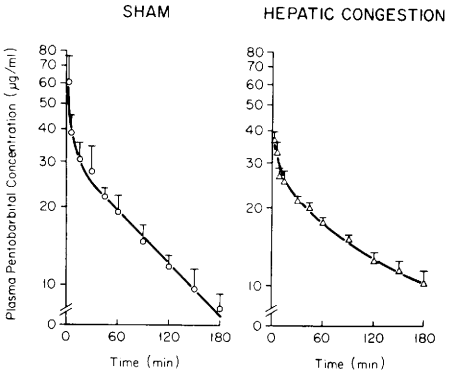

FIG. 2. Composite pentobarbital plasma disappearance curves in sham-operated controls and experimental animals with hepatic congestion. Each point represents the mean plasma pentobarbital concentration ± SEM for that time interval in four animals.

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