Chapter 3

Material and Methods

Studies were carried out on male Wistar rats weighing between 280 and 450 g.

Animal preparation for biliary obstruction

A rat was weighed, anesthetized by intraperitoneal injection of sodium pentobarbitol (Sagatol), 50 mg/kg body weight. Then it was ventrally shaved, swabbed with a solution of alcohol and iodine, and a 2-cm laparotomy made just below the xiphoid process. The common bile duct was double ligated 2 mm apart with thread (Figure B). For the sham control rat, the common bile duct was exposed and cleared without biliary obstruction. Peritoneum, abdominal muscles and skin were sewn separately. The anesthetized rats were warmed up until they awoke, and placed in their cage with free access of food and water.

Operating procedure for renal clearance study

Five days after the common bile duct was ligated, the animal was weighed, then anesthetized again by intraperitoneal injection of sodium pentobarbitol 50 mg/kg body weight. Small supplemental doses were given when necessary. The surgical procedures included a tracheostomy, right carotid artery, left jugular vein and urinary bladder cannulation (Figure C). The tracheostomy was performed by using a short piece of PE 240 polyethylene tube for aspiration of secretion that might block the respiration under anesthetic condition.

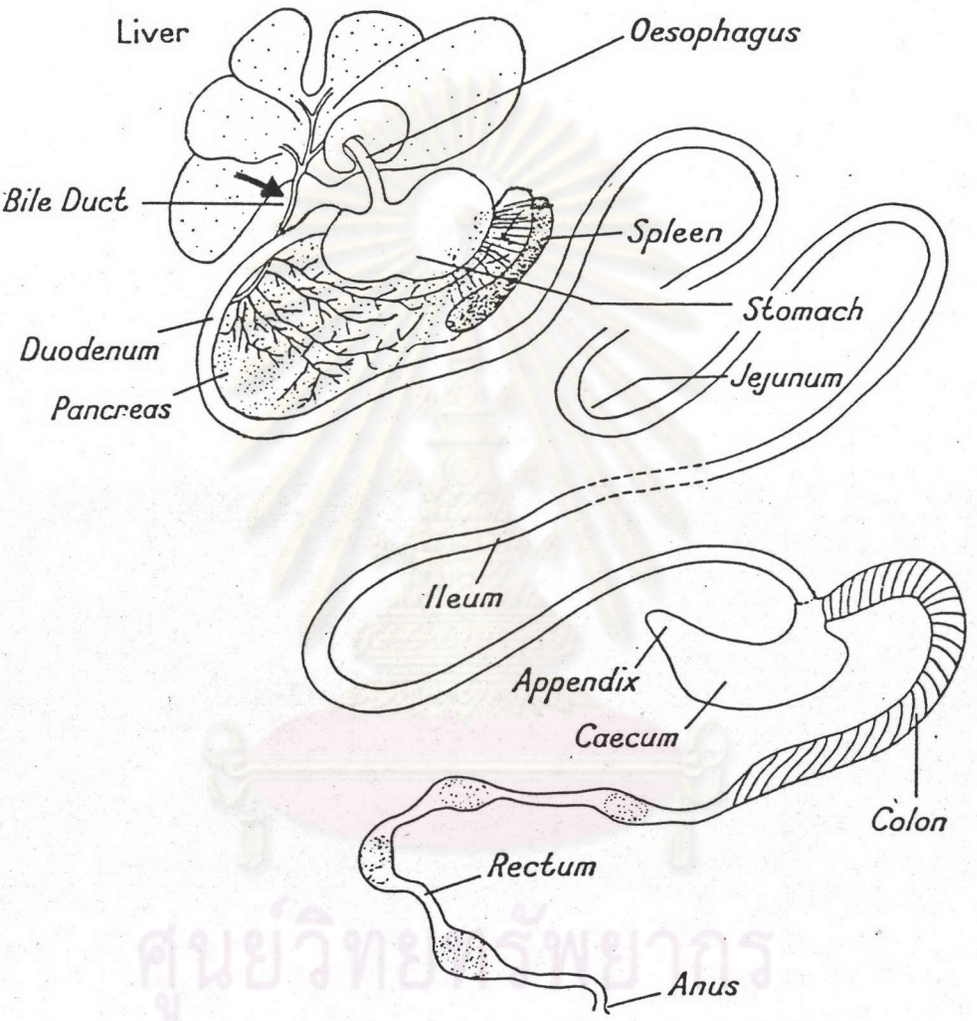

FIGURE B THE POSITION OF BILE DUCT LIGATION

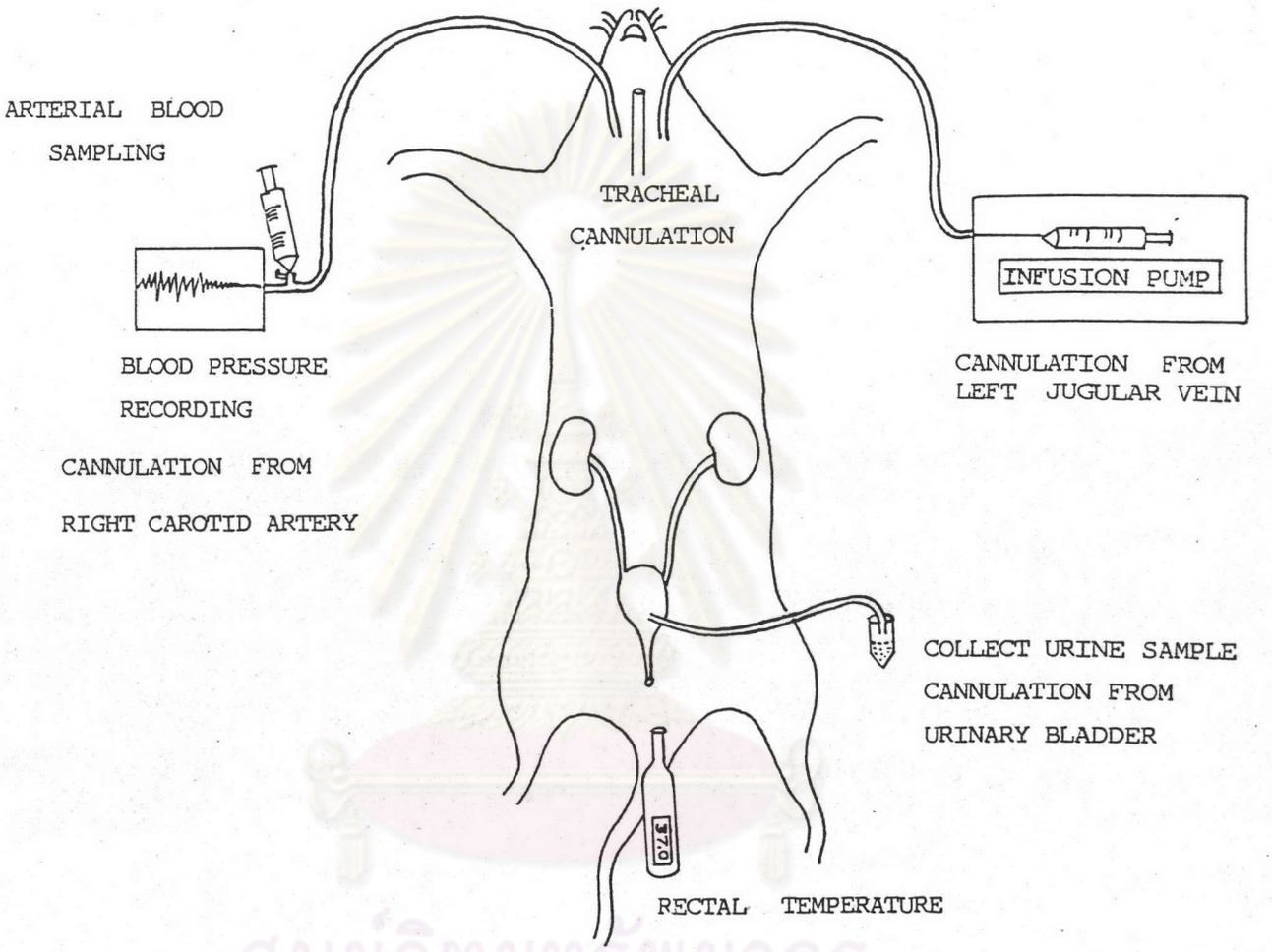

FIGURE C OPERATING PROCEDURE FOR RENAL CLEARANCE STUDY

The right carotid artery was cannulated with a PE 50 polyethylene tube for continuous measurement of arterial blocd pressure (ABP) and for blood sampling. The arterial blood pressure was monitored with a pressure transducer (Statham PE 23 AA) and recorded on a Grass polygragh (Model 7) recorder. The cannulation of left jugular vein was carried out by using a PE 50 polyethylene tube for intravenous infusion. The urinary bladder was inserted with a PE 100 polyethylene tube for urine collection. Urine was collected in a pre-weighed urine cup. The duration of urine collection was between 10 and 30 min depending on the urine flow rate. The urine flow rate was estimated from the change in the weight of the urine cup devided by the duration of the urine collection. The blood sample was taken from the right carotid artery at the end of the period of the urine collection. The heparinized blocd sample was centrifuged and plasma was kept at 4^0C for chemical analysis. Hematocrit was also determined.

Protocol

Male rats were divided into 5 groups.

Group I : Fourteen sham control rats

Group II : Five sham control with vehicle-treated rats (bicarbonate solution $NaHCO_3$ 7.5 g%)

Group III : Eleven sham control with indomethacin-treated rats

Group IV : Eleven biliary obstruction without indomethacin-treated rats

Group V : Ten biliary obstruction with indomethacin-treated rats

Group I : Sham control rats

The common bile duct was exposed and cleared without biliary obstruction. After five days, the rat was prepared for clearance study by the procedure described above. The intravenous infusion of 0.75 mg% inulin and 0.12 mg% PAH in normal saline solution was started at the rate of 0.07 ml/min and kept constant throughout the experiment. After 45 min of the infusion urine and blocd samples were collected.

Group II : Sham control with vehicle-treated rats (bicarbonate solution)

This group of rats was prepared as described in group I. After 45 min, the 7.5 g% bicarbonate solution as a vehicle was infused intravenously as bolus dose (1 ml/kg). After the bicarbonate solution infusion, urine samples were collected. Blocd samples were obtained at the end of the urine collection.

Group III : Sham control with indomethacin-treated rats

This group of rats was prepared as described in group I. After 45 min, the indomethacin solution (5 mg in 1 ml of 7.5 g% bicarbonate solution), was administered intravenously as bolus dose (1ml/kg). After given indomethacin, urine collections were performed in a 10 to 15 min duration. Blood samples were obtained at the end of the urine collections.

Group IV : Biliary obstruction without indomethacin-treated rats

Five days after the preparation for the biliary obstruction,

the rat was prepared by the similar procedure described in Group I.

Group V : Biliary obstruction with indomethacin-treated rats

Five days after the preparation for the biliary obstruction, the rat was prepared by the similar procedure described in Group III.

At the end of each experiment, the rat was injected intravenously with saturated $MgSO_4$. Both kidneys were excised, cleared of perirenal fat, decapsulated and weighed.

Determination of blood and urine samples

Total and direct plasma bilirubin were determined by diazo method as described by Jendrassik and Grof (1938). Inulin and PAH concentrations in plasma and urine were determined by an anthrone method as described by Davidson et al (1963), and by the method of Bratton and Marshall as modified by Smith (1962). Determination of plasma and urine urea nitrogen were carried out by a diacetyl monoxime method as described by Reed et al (1972) and Wybenya et al (1971), calcium by colorimetric method of Moorehead and Biggs (1974) and inorganic phosphorous by the method of Gomori (1941). Osmolarity, sodium, potassium, chloride concentrations in both plasma and urine were determined by Advanced Osmometer (Model 3MO), Beckman flame photometer (Model KLiNa) and Corning Chloride Analyzer (Model 925), respectively. Hematocrit was determined by preparing blood in an international microcapillary tube and then centrifuged by Adam micro hematocrit centrifuge (Model 850) and determined by Hawksley micro hematocrit reader.

Calculation :

Mean arterial blood pressure = Pd + 1/3 (Ps-Pd)

$$\text{Effective renal plasma flow} = \frac{U_{PAH} \times V}{P_{PAH}}$$

$$\text{Effective renal blood flow} = \frac{\text{ERPF} \times 100}{100-\text{Hct}}$$

$$\text{Glomerular filtration rate} = \frac{U_{in} \times V}{P_{in}}$$

$$\text{Filtration fraction} = \frac{\text{GFR} \times 100}{\text{ERPF}}$$

$$\text{Renal vascular resistance} = \frac{\text{MABP} \times 1333 \times 60}{\text{ERBF} \times 1000}$$

$$\text{Urea nitrogen clearance} = \frac{U_{un} \times V}{P_{un}}$$

Excretion rate of electrolyte = Ue X V

$$\text{Fractional electrolyte excretion} = \frac{\text{Ue} \times \text{V/Pe} \times 100}{\text{GFR}}$$

$$\text{Osmolar clearance} = \frac{U_{osm} \times V}{P_{osm}}$$

$$\text{Free water clearance} = V - Cosm$$

Data analysis

Data analysis was done with SPSSPC+ program (Statistical Package for the Social Sciences) and the results were presented as mean $\pm$ one standard deviation (S.D.). These data were statistically compared by Unpaired t-test among the groups. Results considered statistically difference had p-value less than 0.05.