CHAPTER III

MATERIALS AND METHOD

Animals and Procedures

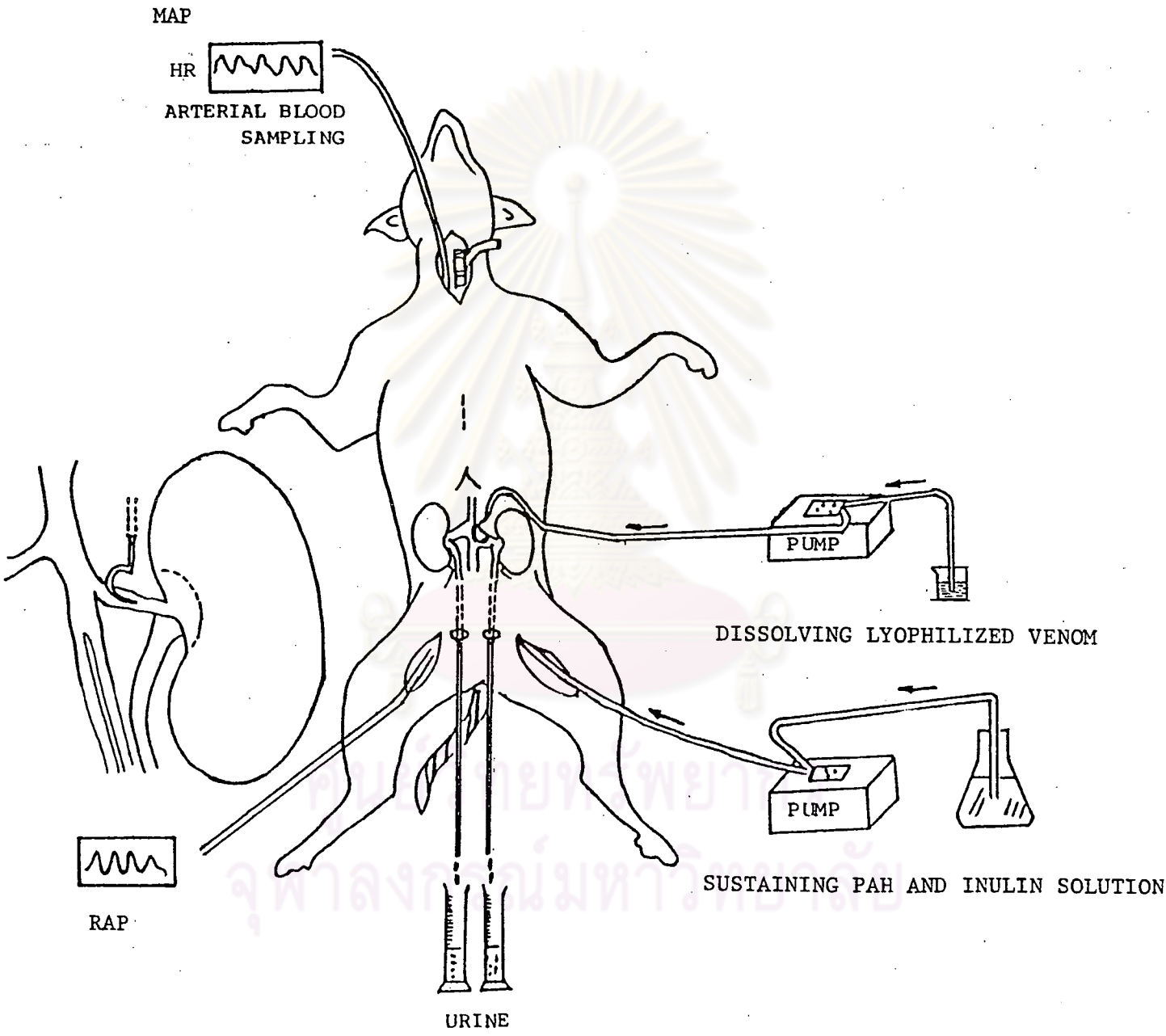
This investigation was conducted on 26 adult male mongrel dogs, weighing 8 to 15 kg. All dogs were maintained on a standard dog chow and tap water ad libitum, kept on for at least 7 days until the time of experiment and were withheld food and water for 12 hours before the experiment. Eight dogs were used for the preliminary study relevant to investigate for the proper dose of the Russell's viper venom. The experimental study was done on eighteen dogs.

The Preliminary Study

Each animal was anesthetized with an initial dose of sodium pentobarbital 25 mg/kg bw. and approximately 5 mg/kg bw. were given throughout the experiment as required to maintain a relatively constant level of anesthesia. The trachea was cannulated with a tracheotomy tube so as to facillitate respiration and removal of excess secretion. The animals were allowed to ventilate spontaneously in room air. The left carotid artery was cannulated with polyethylene tube (PE 240) for collecting blood samples and connected to the pressure tranducer (Statham P 23 AA.) for recording systemic arterial blood pressure and heart rate on a Beckman dynograph (type RM). A polyethylene tube (PE 240) was inserted into the right femoral vein

FIGURE I

SCHEMA OF THE EXPERIMENT



for administration of an isotonic saline solution and additional anesthetic. The left femoral vein was cannulated with a polyethylene tube (PE 240) for infusion of inulin and PAH (P - aminohippuric acid) solutions. The left kidney was exposed retroperitoneally through a flank incision. The left renal artery and ureter were carefully isolated and freed from its attachment without damaging the renal nerve. The polyvinyl tube (PV 200) was introduced into the left ureter for collecting urine samples. A curved 23 - gauge needle attached to polyethylene tube (PE 50) was inserted at the origin of the left renal artery for administration of dissolved venom. During surgical and experimental periods, the animals were infused with isotonic saline solution at a rate of 5 ml/kg bw./hr in order to maintain the body fluid volume. The Russell's viper venom was prepared by dissolving lyophilized venom with 5 ml of an isotonic saline solution. At the end of surgical procedures, the priming solution containing 10 mg/kg bw. of PAH and 50 mg/kg bw. of inulin in an isotonic saline solution was administered intravenously and immediately afterward by the sustaining infusion at the rate sufficient to maintain the plasma PAH and inulin concentration at approximately 0.02 mg/ml and 0.2 mg/ml, respectively. A following period of 50 minutes was allowed for a stabilization of plasma inulin and PAH concentrations. Prior to venom infusion, two blood and urine control samples were obtained via the left carotid artery and ureter, respectively. An arterial blood samples were obtained at the midpoint of each urine collection period. In each dog, the effect of a single dose of dissolved venom was studied on renal hemodynamics by microinfusion pump (Sage Instruments, model 375 A) at the rate of

0.08 ml/kg bw./min varied from 1.0, 0.5, 0.1, 0.05 mg/kg bw. Again, blood and urine samples were obtained at a period of $\frac{1}{2}$, 1, 2, 3, 4, 5 hours, respectively. These were determined for clearance studies of inulin, PAH, BUN, Cr, electrolytes and osmolality. Blood samples were also measured for packed cell volume. These data were evaluated for further studies in the experimental study.

The Experimental Study

This experiment was performed to study the effect of Russell's viper venom on renal hemodynamic and histopathological changes. First part of surgical procedures were performed in the same manner as in the preliminary study. The additional procedures were done. A polyethylene tube (PE 240) was introduced into the left femoral artery and advanced into the abdominal aorta just below the left renal artery for recording approximately renal arterial pressure (Chomdej, 1975). Right ureter was exposed through a retroperitoneal flank incision and freed from the adjacent tissue. Polyvinyl tube (PV 200) was inserted into the right ureter for collecting urine samples. The free ends of both ureteral catheters were tunneled through a small midline incision at the abdominal wall and securely tied. According to the preliminary study, the proper dose for detectable phase changes was 0.05 mg/kg bw. The high comparative dose of 0.1 mg/kg bw. was also studied.

Calibration of Instruments

Systolic and diastolic blood pressure, heart rate were

recorded on Beckman Dynograph recorder (type RM). Using Statham P 23 AA pressure transducer coupled to strain gauge coupler (type 9872) with Beckman preamplifier. Pressure transducers were periodically calibrated against a mercury manometer. Urine samples were collected in graduated centrifuge tubes that were accurate to within 2%. Urine flow rate was computed from the measured volume and time for collection.

The Experimental Period

After the control period, lyophilized venom in either 0.05 or 0.10 mg/kg bw. was dissolved in 5 ml of isotonic saline solution and infused directly into the left renal artery by the rate 0.08 ml/kg bw. /min. The experimental periods were carried out at $\frac{1}{2}$, 1, 2, 3, 4, 5 hours respectively. During the infusion of the dissolved venom, the contralateral kidney was used to compare. At the end of experiment, both kidneys were excised, stripped of surrounding fat and tissue, blotted dry and weighed. For pathological study, kidney tissues were preserved in 10% formalin solution and embedded in paraffin. Most of paraffin sections were stained with hematoxylin-eosin and periodic acid schiff staining was occasionally used. The normal control group was also done but infused 5 ml an isotonic saline solution instead of dissolved venom.

The Experimental Protocol

The animals were divided into three groups as followed :

group 1. served as normal controls. Five dogs were infused with 5 ml an isotonic saline solution directly into left renal artery.

group 2. After control clearance studies, the dissolved venom of 0.05 mg/kg bw. was administrated in 8 dogs.

group 3. Using high comparative dose of 0.1 mg/kg bw. was infused constantly through left renal artery in 5 dogs. The histopathological studies were performed in all groups.

Analytic Techniques

Determination of inulin in plasma and urine was carried out according to the method of Schreiner as described by Smith (1962).

PAH concentration in plasma and urine were determined with the method of Bratton and Marshall as modified by Smith (1962).

The concentrations of sodium and potassium in plasma and urine were measured by a flame photometer (Instrumentation Lab., model 343) and chloride were measured by a chloride analyzer (Instrumentation Labs., model 279). Plasma and urine osmolarity were determined using the freezing point depression method (Advanced Instrument Osmometer, model 3D). Packed cell volume was prepared by microcapillary tube and then centrifuged by microcapillary centrifuge (Runne Heidelberg, model 85-1). Blood urea nitrogen was measured by Diacetyl monoxim. Plasma and urine creatinine were determined with Jaffe reaction.

Using Fick's principle, PAH clearance was used for measuring effective renal plasma flow (ERPF) and inulin clearance was used for measuring glomerular filtration rate (GFR).

Calculation

Mean systemic or renal arterial blood pressure

$$= Pd + \frac{1}{3}(Ps - Pd)$$

Pd = diastolic blood pressure

Ps = systolic blood pressure

Renal vascular resistance $= \frac{RBP}{ERBF}$

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

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

Effective renal blood flow $= \frac{ERPF \times 100}{(100-PCV)}$

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

Osmolar clearance $= \frac{U_{Osm} \cdot V}{P_{Osm}}$

Free water clearance $= V - C_{Osm}$

Urinary electrolytes excretion $= U_e \cdot V$

$$\text{Fractional electrolytes excretion} = \frac{U_e \cdot V/P_e}{GFR} \times 100$$

$$\text{Filtered load of electrolytes} = P_e \times GFR$$

$$\text{Ratio of } U/P_{Osm} = \frac{U_{Osm}}{P_{Osm}}$$

Analysis of Data

Experimental data were expressed as mean ± SEM. Statistical significance was tested according to Student's t-test for paired or unpaired variates. P-values less than 0.05 ($P < 0.05$) were accepted as being statistically significant.

Abbreviations and derivation of variables used in text and figures.

MAP = mean arterial blood pressure (mm.Hg)

RAP = mean renal arterial blood pressure (mm.Hg)

HR = heart rate (beat/min)

PCV = packed cell volume (%)

RVR = renal vascular resistance (mm.Hg/ml/min)

V = urine flow rate (µl/min/gm - kidney weight)

P_{in} = plasma concentration of inulin (mg/ml)

U_{in} = urinary concentration of inulin (mg/ml)

C_{in} = inulin clearance (ml/min/gm - kidney weight)

P_{PAH} = plasma concentration of PAH (µg/ml)

U_{PAH} = urinary concentration of PAH (µg/ml)

C_{PAH} = PAH clearance (ml/min/gm - kidney weight)

ERPF = effective renal plasma flow (ml/min/gm - kidney weight)

RBF = renal blood flow (ml/min/gm - kidney weight)

P_{Osm} = plasma osmolality (mOsm/kg)

U_{Osm} = urinary osmolality (mOsm/kg)

C_{Osm} = osmolar clearance (µl/min/gm - kidney weight)

C_{H_2O} = free water clearance (µl/min/gm - kidney weight)

P_{Na} = plasma concentration of sodium (mEq/L)

U_{Na} = urine concentration of sodium (mEq/L)

P_{Cl} = plasma concentration of chloride (mEq/L)

U_{Cl} = urine concentration of chloride (mEq/L)

P_{K} = plasma concentration of potassium (mEq/L)

U_{K} = urine concentration of potassium (mEq/L)

P_{Cr} = plasma creatinine (mg %)

BUN = blood urea nitrogen (mg%)