CHAPTER III

MATERIALS AND METHODS

Animals and Procedures

Experiments were carried out on twenty-five adult male mogrel dogs weighting 12-18 kgs. All dogs were maintained on a standard dog chow and tap water ad libitum and kept for at least 7 days until the time of experiment. The animals were fasted for 12 hours preceeding an operation. Ten dogs were used for pilot study and experimental study was done on fifteen dogs.

General Procedure

Each animal was anesthetized with an initial dose of sodium pentobarbital 25-30 mg/kg-bw and approximately 5 mg/kg-bw was given throughout the experiment as required to maintain a relative constant level of anesthesia. the trachea was cannulated with tracheostomy tube so as to facillitate respiration and removal excess secretion. The animal were allowed to ventilate spontaneously in room air. A polyethylene tube (PE 240) was inserted into right femoral vein for infusion of an isotonic saline solution to maintain extracellular fluid volume, inulin and PAH (para-aminohippuric acid) solutions. The left femoral vein was cannulated with polyethylene tube (PE 240) for administration of an isotonic saline solution, 0.3 N

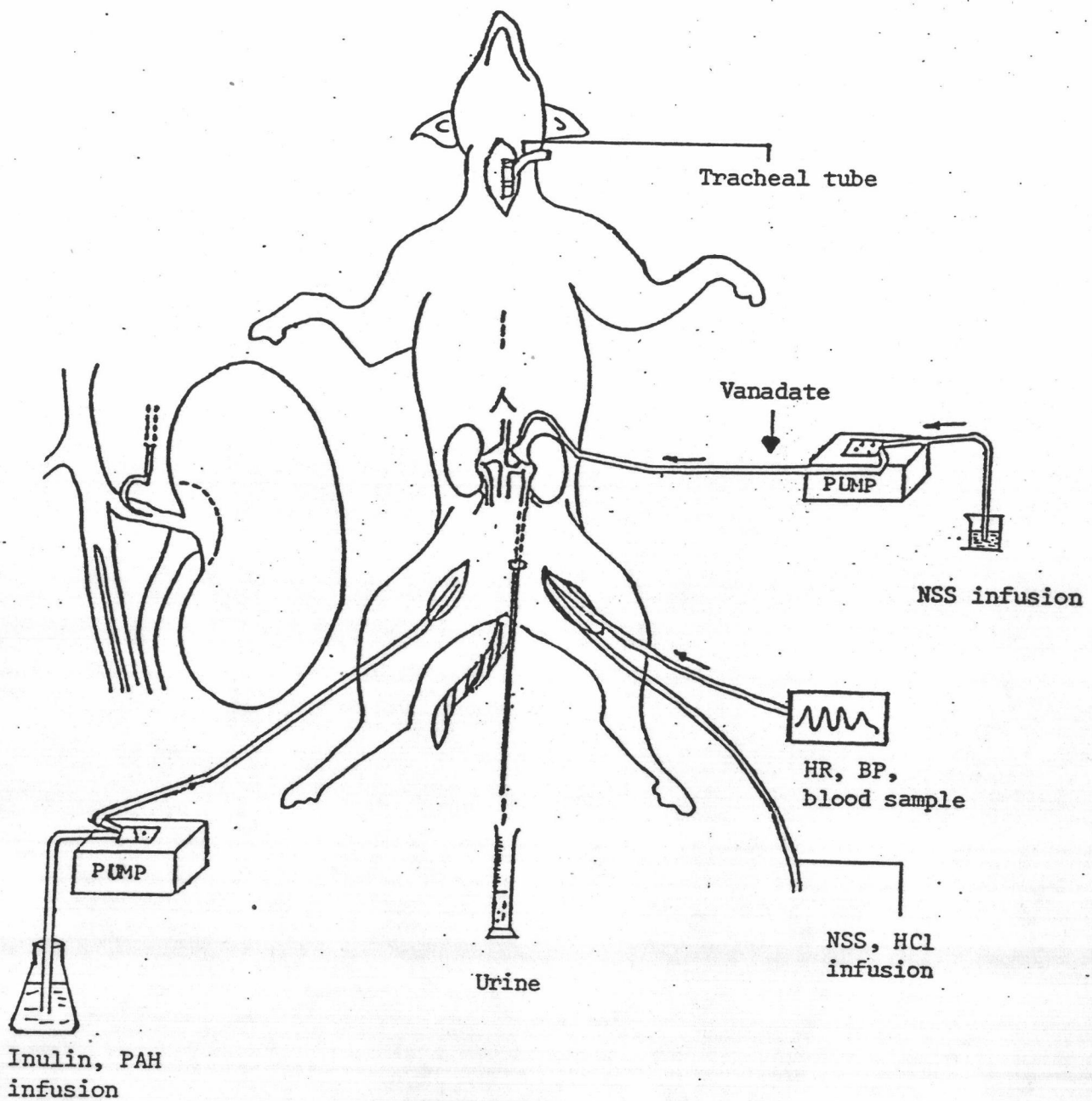

Fig. 1 Scheme of Experiment

hydrochloric acid and addition anesthetic. The left femoral artery was also cannulated for collecting blood sample and connected to the pressure transducer (elcomatic EM 750) for recording systemic arterial blood pressure and heart rate on a Harvard universal oscillograph (Model 50-9307). The left kidney was exposed retroperitoneal through a flank incision. The left renal artery and ureter were carefully isolated and freed from its attachment without damaging the renal nerve. The polyethylene tube (PE 200) was introduced into the left ureter for collecting urine samples. A curved 23-gauge needle attached to polyethylene tube (PE 250) was inserted at the origin of the left renal artery for infusion of an isotonic saline solutions by connecting with the continuous automatic infusion pump (Harrard, Model 600-954) at the rate of 0.5 ml/kg/hr and bolus dose of dissolved sodium orthovanadate. During surgical and experimental periods, the animals were infused with isotonic saline solution at a rate of 5 ml/kg/hr in order to maintain the body fluid volume. The vanadate (sodium orthovanadate) was prepared by dissolving with 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 followed by the sustaining infusion at the

rate sufficient to maintain the plasma PAH and inulin concentrations at approximately 0.02 mg/ml and 0.2 mg/ml, respectively. A following period of 45 minutes was allowed for a stabilization of plasma inulin and PAH concentrations. Prior to 0.3 N hydrochloric acid and vanadate administration, two control blood and urine samples were obtained via the left femoral artery and ureter, respectively. An arterial blood sample were obtained at the mid point of each urine collection period. There were determined for inulin, PAH, and electrolytes. Blood samples were also measured for creatinine and hematocrit. Urine samples were measured for ammonium ion and titratable acid.

Calibration of Instruments

Heart rate, systolic and diastolic blood pressures were recorded on Harvard universal oscillograph (Model. 50-9307).

Pressure transducers were periodically calibrated against a mercury manometer. Urine samples were collected in graduated centrifuge tube that were accurated to within 2% and under paraffin oil. Urine flow rate was computed from the measured volume and time collection.

Experimental Periods

After the control period, in acute metabolic acidosis , continuous infusion of the 0.3 N hydrochloric acid was administered at a rate of 3 mEq/kg/hr. During each experimental period, two timed urine volumes were collected and blood sample was taken at the mid point of each urine sample. After the collection of two timed urine and blood sample, the injection of vanadate solution 1.43 µmol/kg was administered directly into the left renal artery and then collected two timed urine and blood sample. After the control period, in saline control vanadate solution was also injected 1.43 µmol/kg into the left renal artery.

At the end of experiment, the kidney was excised, stripped of surrounding fat and tissue, blotted dry and weighed so that renal blood flow and glomerular filtration rate could be expressed as milliliters per minute per gram of kidney weight.

The Experiment Protocol

The animals were divided in two groups :

group 1 : Saline control. Five dogs were given continuously an intravenous infusion of isotonic saline solution and bolused with vanadate solution 1.43 μmol/kg directly into left renal artery

group 2 : Acute metabolic acidosis. Ten dogs were induced to be acute metabolic acidosis by a continuous infusion of 0.3 N hydrochloric acid intravenously at the rate of 3 mEq/Kg/hr, an then bolused with vanadate solution 1.43 μmol/kg directly into left renal artery.

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 was 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).

Plasma and urine concentrations of chloride were measured by a chloride analyzer (Instrumentation Lab., Model 279). Hematocrit was prepared by microcapillary tube and then centrifugued by microcapillary centrifuge (Runne Heidelberg, Model 85-1).

Plasma creatinine was determined with Jaffe reaction. Plasma and urine bicarbonate, ammonium ion (NH_4) and titratable acid (TA) were measured by titration.

PAH and inulin clearance was used for effective renal plasma flow (ERPF) and glomerular filtration rate (GFR), respectively.

Abbreviation

Mean systemic arterial blood pressure

$$= DP + 1/3\ (SP - DP)$$

DP = diastolic blood pressure

SP = systolic blood pressure

Effective renal plasma flow

$$= \frac{U_{PAH} \cdot V}{P_{PAH}}$$

Glomerular filtration rate

$$= \frac{U_{in} \cdot V}{P_{in}}$$

Urinary electrolyte excretion

$$= Ue.V$$

Fractional electrolyte excretion

$$= \frac{Ue \cdot (V/Pe)}{GFR} \times 100$$

Urinary acid excretion

$$= Ua \cdot V$$

Net acid excretion

$$= U_{TA}V + U_{NH_4}V - U_{HCO_3}V$$

Analysis of Data

Experimental data were expressed as mean $\pm$ SEM. Statistical significance was tested according to student's paired t-test, p-values less than 0.05 ($p < 0.05$) were accepted as being statistically significance.

Abbreviations and derivation of variable used in text and figures.

MAP	=	mean arterial blood pressure (mmHg)
HR	=	heart rate (beat/min)
Hct	=	hematocrit (%)
V	=	urine flow rate (ul/min/gm-kidney weight)
P_{in}	=	plasma concentration of inulin (mg/ml)
U_{in}	=	urinary concentration of inulin (mg/ml)
C_{in}	=	plasma clearance of inulin (ml/min/gm-kidney weight)
GFR	=	glomerular filtration rate (ml/min/gm-kidney weight)
P_{PAH}	=	plasma concentration of PAH (ug/ml)
U_{PAH}	=	urinary concentration of PAH (ug/ml)
C_{PAH}	=	plasma clearance of PAH (ml/min/gm-kidney weight)
ERPF	=	effective renal plasma flow (ml/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_{HCO_3} = plasma concentration of bicarbonate (mEq/L)

U_{HCO_3} = urine concentration of bicarbonate (mEq/L)

U_{TA} = urine concentration of titratable acid (mEq/L)

U_{NH_4} = Urine concentration of ammonium ion (mEq/L)

P_{Cr} = plasma concentration of creatinine (mg %)