CHAPTER III

MATERIALS AND METHODS

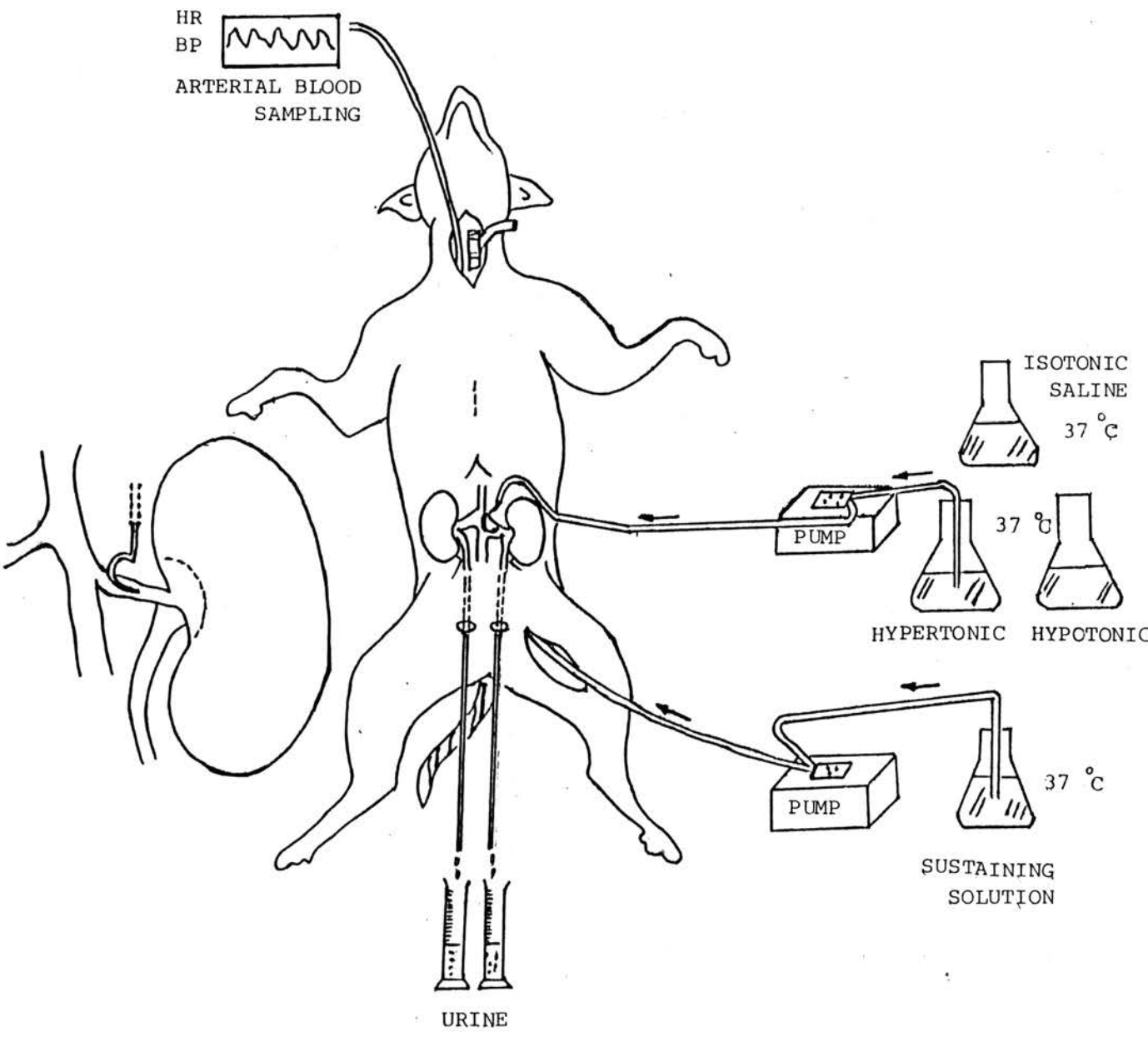
Animals and procedures

The experiments were performed on 17 adult male mongrel dogs, weighing between 10-18 kgs. The dogs were devided into three groups. The animals in group I, II and III were selected as control, hypothyroid and hyperthyroid groups respectively. The experiments consisted of a pre-experimental peroid lasting 1 week, followed by the experimental period. In the pre-experimental period, six dogs in group II were surgically thyroidectomized (replaced with 1% $CaCl_2$ in drinking water) and five dogs in group III were received daily subcutaneous injections of L-thyroxine 0.1 mg/kg for 1 week before the experiment (to induce a hyperthyroid state).

Before an experiment, the animals were fasted for 12 hours and anesthetized with pentobarbital sodium 25 mg/kg body weight intravenously and were subsequently given small maintenance dose as necessary. The tracheostomy was performed and the animals were allowed to ventilate spontaneously in room air. Polyethylene catheter (PE 200) was cannulated into the common carotid artery for recording the arterial blood pressure (BP), heart rate (HR) and for collecting blood samples. The left femoral vein was cannulated with polyethylene tube (PE 190) for infusion of inulin, PAH and injection of Evan's blue (T-1824). Both ureters and one renal artery were exposed

FIGURE A

SCHEMA OF THE EXPERIMENT



by right and left high paracostal incisions. Both ureters were isolated form the surrounding connective tissue and catheterized with polyvinyl tube (PV 200). The free ends of both tubes were tunneled through a small midline incision in the abdominal wall and securely tied for urine collection.

Random sampling of the right or left renal artery and freed from the surrounding connective tissue by careful dissection to preserve the renal nerves. Hooked needle(22 gauge) with polyethylene tube (PE 50) was introduced into that renal artery and left it in place throughout the experiment. The hooked renal artery was perfused constantly with isotonic saline solution by microinfusion pump (Eyela Model MP 3) at the rate of 2.0 ml/min. The rate of infusion is similar to the report by earlier investigators (Chaiyabutr and Malila, 1977).

After the surgical procedures had been finished, the priming solution containing 8 mg/kg of body weight of p-aminohippuric acid (PAH) and 50 mg/kg of body weight of inulin in normal saline was administered intravenously via femoral vein followed immediately by the sustaining solution infusion at the rate of 1.5 ml/min by microinfusion pump (Model MP 3) to maintain the plasma inulin and PAH concentrations by approximately 0.2 mg/ml and 0.02 mg/ml respectively. The rate of infusion was kept constant throughout the course of the experiment.

A period of $1\frac{1}{2}$ hours of infusion was allowed for a stabilization of plasma inulin and PAH concentrations. After the urine flow

rate was stable, the blood and urine samples were collected. 10 minute clearance studies were carried out simultaneously from both kidneys. An arterial blood sample was collected form the carotid artery at the midpoint of each 10 minute urine collection, for inulin, PAH, osmolarity, sodium, potassium, chloride, calcium and inorganic phosphorus. A urine specimen was collected separately from each ureter at the each of 10 minute period to determine the same parameters of blood sample. Packed cell volume was also determined by the arterial blood sample.

Either the hypertonic saline solution (537 mOsm/kg) or the hypotonic saline solution (27 mOsm/kg) was infused directly into the right or the left renal artery at a rate of 2.0 ml/min instead of the isotonic saline solution (290 mOsm/kg). During the infusion of one kidney with saline solution, the contralateral kidney was used to compare with the other one.

Protocol of the experiment :

Immediately after the priming dose, the infusions of the sustaining solution of PAH and inulin were given by the femoral vein while one renal artery was administered with isotonic saline . $1\frac{1}{2}$ hours later, plasma and urine samples were taken continuously following the diagram :

Elapse time (minute)	The infused kidney*	The contralateral kidney
0 - 10	Isotonic saline	control
10 - 20	Hypertonic saline	control
20 - 30	Isotonic saline	control
30 - 90	Isotonic saline solution was infused and enough time was allowed for the urine flow rate in the experimental kidney to return to control rate	control
90 - 100	Isotonic saline	control
100 - 110	Hypotonic saline	control
110 - 120	Isotonic saline	control

(* Side of treatment was selected at random).

Chemical methods :

Cardiac output (CO) and plasma volume (PV) determinations were using T-1824 according to dye dilution technique (Evan's blue, T-1824) as described by Chaiyabutr (1980). Measurement of the plasma volume was determined by the method of Kolmer (1951).

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

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

Routine measurements of sodium and potassium concentrations in plasma and urine were determined by Flame photometer (Beckman, Klina Flame), chloride by chloridometer (Buchler, Digital chloridometer), calcium by the method of cresolphthalein complexone (Moorehead and Biggs, 1974), inorganic phosphorus was carried out according to the method of Gomori (1942), the plasma thyroxine (T_4) by the method of RIA, osmolarity by using the freezing-point depression method (Osmometer, Model 3W), packed cell volume by the preparation of the blood in an International microcapillary centrifuge (Clay-Adams).

Calculations:

The following symbols are used :

V = urine flow rate (ml/min)

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

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

C_{in} = inulin clearance (ml/min)

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

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

C_{PAH} = PAH clearance (ml/min)

P_{Osm} = plasma osmolarity (mOsm/L)

U_{Osm} = urine osmolarity (mOsm/L)

C_{Osm} = osmolar clearance (ml/min)

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

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

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

U_K = urine concentration of potassium (mEq/L)

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

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

P_{Ca} = plasma concentration of calcium (mg %)

U_{Ca} = urine concentration of calcium (mg %)

P_{Pi} = plasma concentration of inorganic phosphorus (mg %)

U_{Pi} = urine concentration of inorganic phosphorus (mg %)

CO = cardiac output (L/min)

PV = plasma volume (L)

Using the Fick Principle, PAH clearance was used to measure effective renal plasma flow (ERPF) and inulin clearance was used to measure glomerular filtration rate (GFR). The following calculations were performed :

Glomerular filtration rate (GFR) = $C_{in} = \frac{U_{in} \cdot V}{P_{in}}$

Effective renal plasma flow (ERPF)= $C_{PAH} = \frac{U_{PAH} \cdot V}{P_{PAH}}$

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

Free water clearance (C_{H_2O}) = $V - C_{Osm}$

Urinary excretion of electrolytes = $U_E \cdot V$

Fractional excretion of electrolytes (%) = $\frac{U_E \cdot V / P_E \times 100}{GFR}$

Renal blood flow (RBF) = $\frac{ERPF}{1-Hct}$

Filtration fraction (FF) = $\frac{GFR}{ERPF}$

$$\text{Renal fraction (\%)} = \frac{\text{RBC} \times 100}{\text{CO}}$$

$$\text{RVR (dyne-sec/cm}^5\text{)} = \frac{\text{mean blood pressure} \times 1333 \times 60}{\text{RBF}}$$

$$\text{TPR (dyne-sec/cm}^5\text{)} = \frac{\text{mean blood pressure} \times 1333 \times 60}{\text{CO}}$$

Statistical analysis

Data was processed according to the pair and unpaired t-test, P-value less than 0.05 was considered significantly.