CHAPTER II

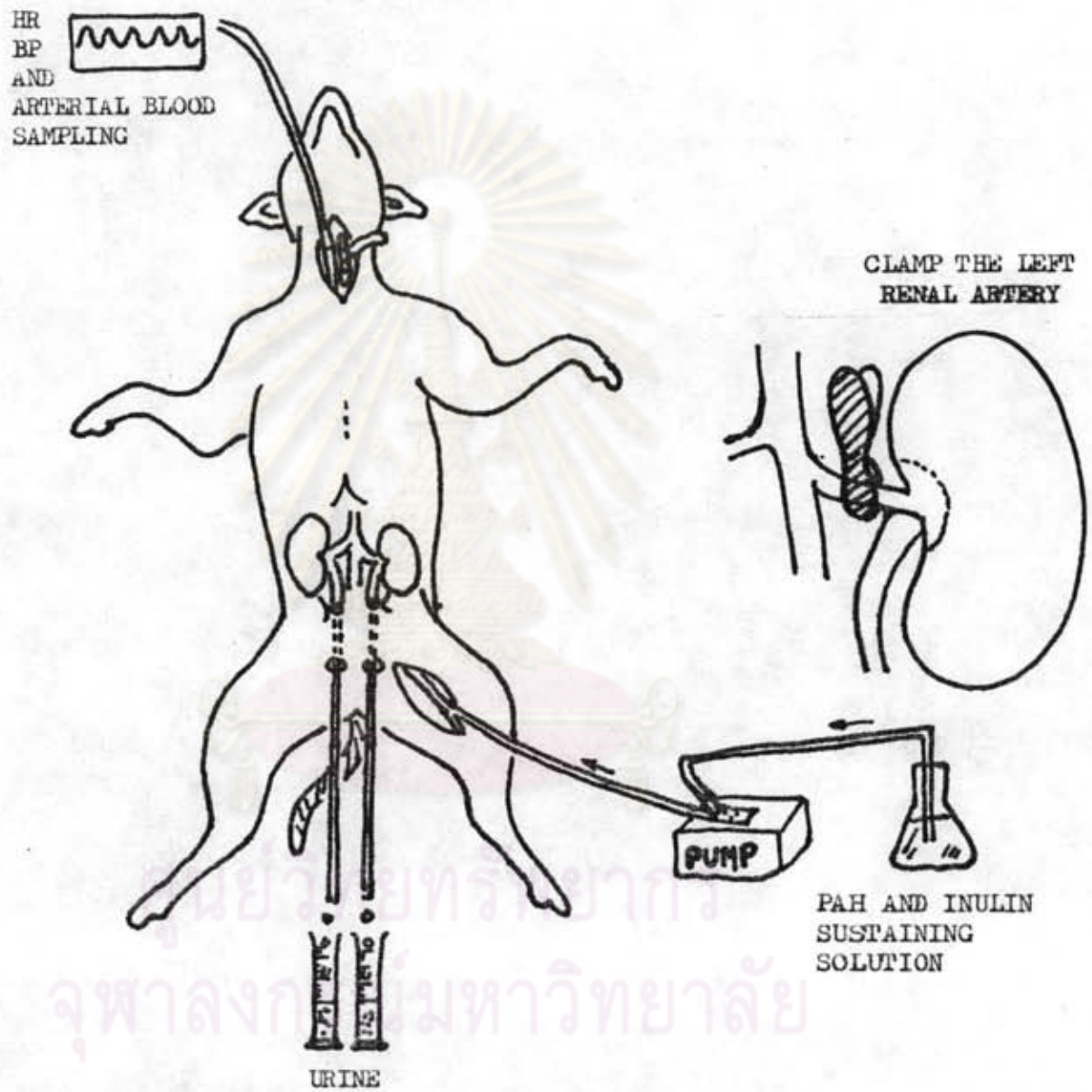
MATERIALS AND METHODS

The experiments were performed on 12 adult male dogs weighing between 9-17 kilograms (kg). All dogs were fasted for 12 hours (hr) before the experiment. They were anesthetized with pentobarbital sodium at a dose of approximately 25 mg/kg body weight intravenously. Small supplemental doses were given as needed during the experiment to maintain anesthesia. The tracheal tube was inserted by tracheostomy 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 collect blood samples. The left femoral vein was cannulated with polyethylene tube (PE 190) for infusion of inulin, PAH and for injection of Evans blue (T-1824). Both ureters were isolated by left and right flank incisions and were freed from the surrounding connective tissue. They were catheterized with polyethylene tubes (PE 190) for urine collection. Two groups of experiment were carried out ; first (group I) to study the effect of hypertonic saline infusion on ischemic-ARF, the second (group II) to study the effect of hypertonic saline infusion on $HgCl_2$-induced ARF. (Figure 1)

GROUP I

Six male dogs were prepared for the first group of experiment. In addition, both ureters were exposed by left and right flank incisions

FIGURE 1 SCHEMA OF THE EXPERIMENT



The left kidney was induced ARF by temporary clamping the left renal artery for 45 minutes while the right kidney was set for control. At 30 minutes after releasing clamp, the animal was infused with 20 milliliter of hypertonic saline 7.5 % (2564 mOsm/L) via femoral vein within a minute. Measurement of general circulatory and renal functions were performed before, during clamp 45 minutes and at 10 minutes after hypertonic saline infusion.

GROUP II

Six male dogs were induced ARF by injection of $HgCl_2$ (5 mg/kg) into femoral vein. At 45 minutes after injection, the animal was infused with 20 milliliter of hypertonic saline 7.5 % via femoral vein within a minute. Measurement of general circulatory and renal functions of both kidneys were performed before, 45 minutes after $HgCl_2$ injection and at 10 minutes after hypertonic saline infusion.

In order to maintain the body fluid volume, an isotonic sodium chloride solution was infused at a rate 1.5 ml/min by Eyela microinfusion pump (Model MP 3) during surgical period.

A control samples were taken prior to infusion of 0.5 % of inulin and 0.2 % of p-aminohippuric acid (PAH) in normal saline. For the priming dose, 60 mg/kg of body weight of inulin and 30 mg/kg of body weight of PAH were administered. These were followed by a sustaining infusion at a rate sufficient to maintain the plasma inulin and PAH concentrations at approximately 0.2 mg/ml and 0.04 mg/ml plasma respectively.

A period of 1 hr of infusion was allowed for a stabilization of plasma inulin and PAH concentrations and the rate of urine flow stabilized. The initial blood and urine collections were performed. First 10 minutes clearance studies were carried out simultaneously from both kidneys as in the control period. An arterial blood sample was collected from the carotid artery at the midpoint of each 10 minutes urine collection. Measurements of inulin, PAH, osmolarity, sodium, potassium, chloride concentrations were determined in plasma and urine samples. Blood samples were also determined for packed cell volume (PCV) and hemoglobin (Hb).

Urine collections were performed simultaneously at every 10 minutes interval for chemical determinations. Mean arterial blood pressure (MAP) and heart rate (HR) were recorded on polygraph (Grass Model 7).

Cardiac output (CO) and plasma volume (PV) determinations were using T-1824 : The cardiac output was carried out according to dye dilution technique (Evans 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).

Plasma protein free filtrate was used in determination of PAH and inulin was prepared with cadmium sulphate.

Routine measurements of sodium and potassium concentrations in plasma and urine were determined by Flame photometry (Beckman, Klina flame), chloride by Chloridometer (Buchler, Digital chloridometer), osmolarity by using the freezing-point depression method (Osmometer, Model 3W). Packed cell volume was measured by the preparation of the heparinized blood in microcapillary centrifuge. Hemoglobin was measured by the Cyanmethemoglobin method.

CALCULATIONS

The following symbols are used throughout the calculation.

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, ug/ml
U_{PAH}	=	urine concentration of PAH, ug/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
PCV	=	packed cell volume, %
CO	=	cardiac output, L/min

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

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

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

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

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

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

STATISTICAL ANALYSIS

Data were processed according to Pair t-test and Two way analysis of variance of the same dog. The difference between treatments was tested by using DUNCAN'S NEW MULTIPLE RANGE TEST. A p-value less than 0.05 was considered significant.