Chapter III

Materials and Methods

Animals Preparation

Experiments were performed in 16 male mongrel dogs, weighing 9 - 17 kgs. Two groups of eight animals were used. Intact animals in group I were used as a control group and animals in group II were thyroparathyroidectomized 24 hrs. before start of the experiment. On the day of experiment, the animals were fasted for 12 hours prior to the experiment, but permitted free access to water. They were anesthetized with the intravenous injection of sodium pentobarbital 30 mg/kg.bw initially, and recieved subsequent doses of 1 - 2 mg/kg.bw when necessary to maintain light anesthesia throughout the experiment.

Surgical Procedure

A tracheal cannula was inserted by tracheostomy to secure free airways. Polyethylene catheters (PE 180) were introduced into left femoral artery for blood pressure recordings, into right femoral artery for blood sampling and into femoral vein for inulin and PAH infusion. A pressure transducer (PE 23 AA) and polygraph (Grass Model 7) recorder were used for blood pressure and heart rate estimation.

A paracostal incision was made for retroperitoneal approach to the left kidney. The ureter was drained by indwelling ureteral cathter (PV 190) for urine sampling. A hook shape 23 gauge needle attached to polyethelene catheter (PE 50) was hooked at the base of

renal artery, anterograde to renal arterial flow, for infusion of 0.9% NSS and dissolved Russell's viper venom during control and experimental periods respectively (Fig A).

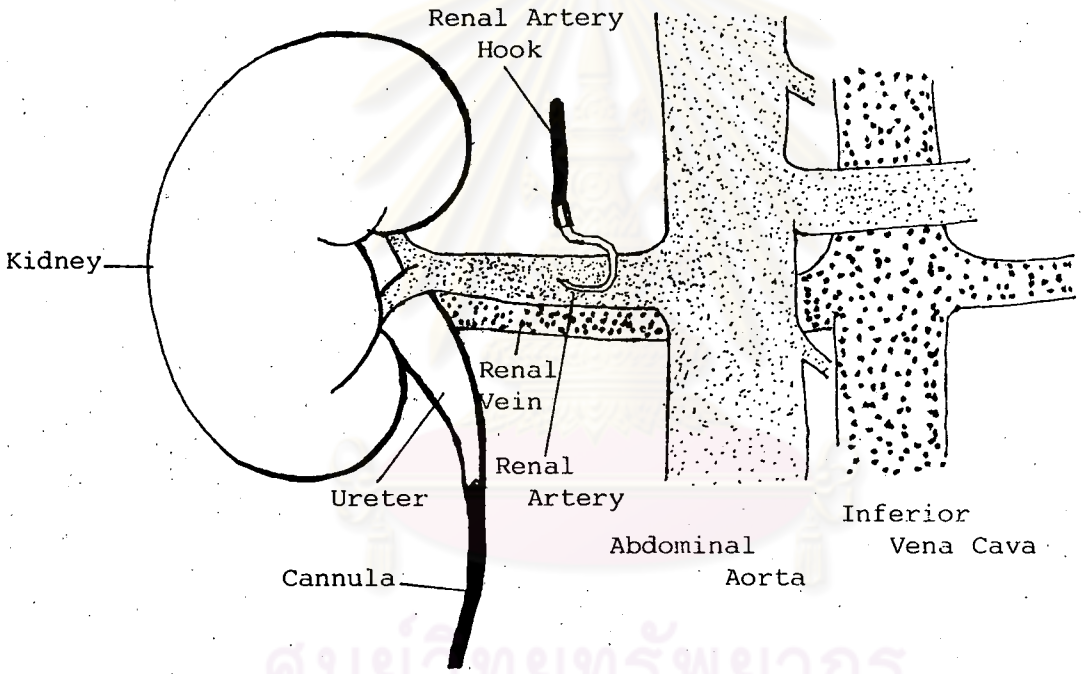
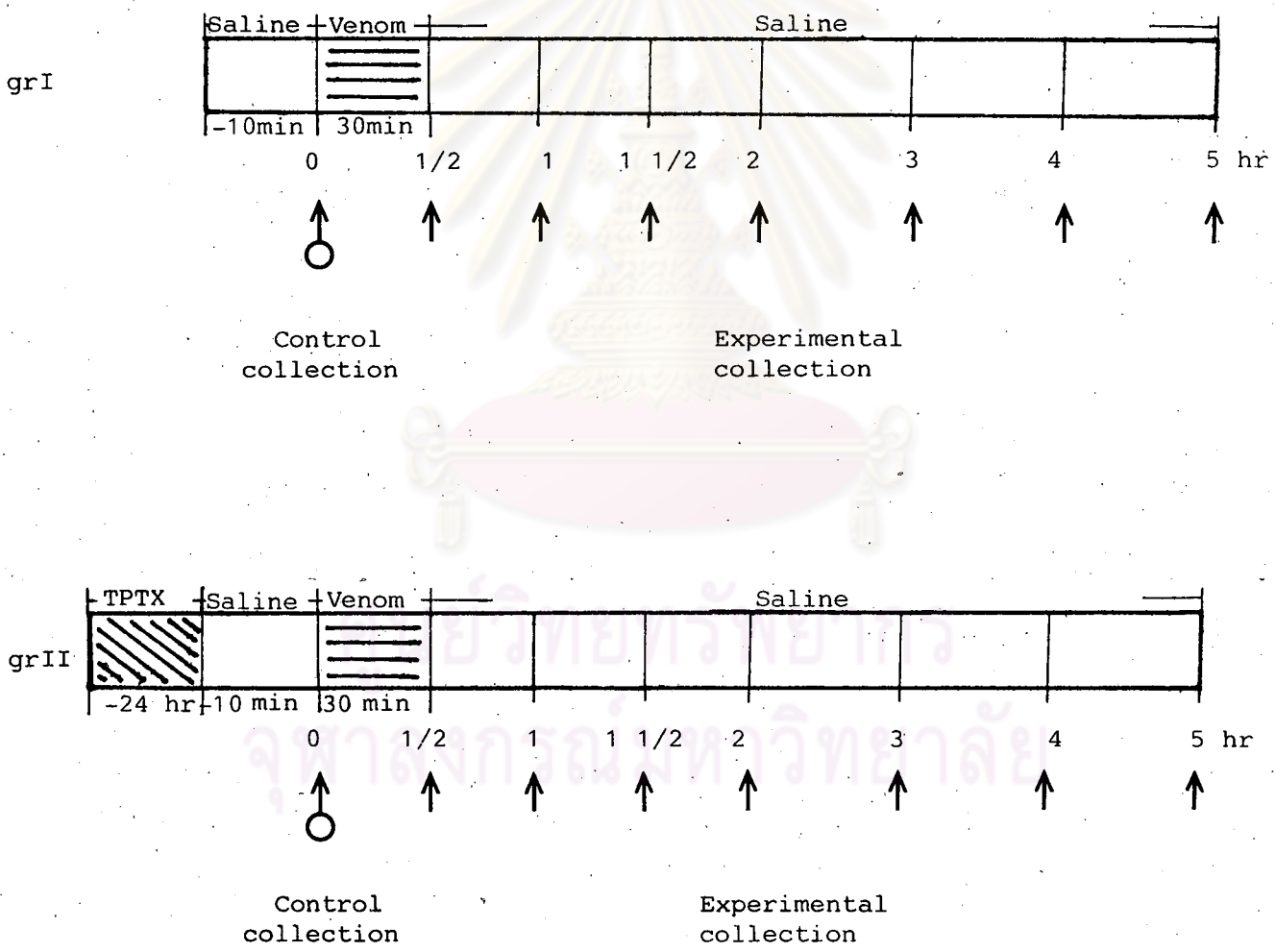


Fig A : A diagrammatic illustration of the technique used for studying renal hemodynamics and renal functions.

Experimental Protocol

Two groups of eight animals were used to study the effects of Russell's viper venom on the renal handling of inorganic phosphorus. Experiments were carried out in both groups as following :



Experimental Procedures

After surgical procedure, animals were then given the priming dose of 25 mg/kg.bw of inulin and 6 mg/kg.bw of PAH dissolved in 0.9 % NSS intravenously and immediately afterward by the sustaining infusion of 500 mg % of inulin and 120 mg % of PAH at the rate of 1.8 ml/min with peristaltic pump (Eyla Model 3), that was sufficient to maintaiin the plasma inulin and PAH concentration at approximately 0.2 mg/ml and 0.02 mg/ml respectively. The pH of dissolved solution for priming and sustaining dose was adjusted to 7.4 with 1 N NaOH solution. A minimum of 60 minutes was allowed for equilibration and stabilization. The systemic blood pressure and heart rate were recorded from left femoral artery by pressure transducer (PE 23 AA) which connected to the polygraph recorder (Grass Model 7). Prior to venom infusion 0.9 % NSS was infused into renal artery by the rate 0.32 ml/min with syringe pump (Sage Instruments Model 341 A). After that blood and urine were sampled for the control (0) period. The dose of 0.02 mg/kg.bw of Russell's viper venom, dissolved in 0.9 % NSS 10 ml, was infused intrarenal arterially with syrige pump at the rate of 0.32 ml/min . The post venom infusion period of 1/2, 1, 1 1/2, 2, 3, 4 and 5 hours were sampled consecutively till the end of experiments. The urinary titratable acid and packed cell volume were measured closely to the end of each sampling period.

Determination of Urinary Titratable Acid (Folin method)

Titratable acidity was estimated by titrating a fresh specimen of urine with 0.01 N NaOH, using 1 % phenolphthalein as an indicator, the end point pH is 8.3. Transfer 500 ul of urine to a test tube and

add 0.35 gm of powdered potassium oxalate ($K\ (COO)_2\ H_2O$) to precipitate the calium which would otherwise interfere with the end-point, since calcium phosphate precipitates on neutralization of the urine. One drop of 1 % phenolphthalein was added into the tube and shook well for 2 - 3 minutes. Place 0.01 N NaOH solution in 5 ml burette and titrate to a pale pink color (pH 8.3). The difference between the burette readings before and after the titration is the volume of NaOH used. The urinary titratable acid excretion was calculated as following :

$$\text{Normality of acid} = \frac{\text{0.01 N NaOH x ml of 0.01 N NaOH used}}{\text{ml of urine (500 µl)}}$$

$$\text{Urinary titratable acid excretion} = \frac{\text{Normality of acid x total urine}}{\text{time collection of urine}}$$

$$= \text{mEq/min of total acid.}$$

Determination of Blood and Urine Samples

Plasma and urine inulin concentrations were determined by the anthrone method as described by Davidson et al (1963). Determination of plasma and urine PAH concentration were carried out by the method of Bratton and Marshall as modified by Smith (1962). Using the Fick's principle, PAH clearance was used for determination of effective renal plasma flow (ERPF) and inulin clearance was used for glomerular filtration rate (GFR).

The sodium and potassium concentrations in plasma and urine were determined by flame photometer (KLiNa flame operating; Beckman instrument), chloride by chloridometer (Buchler digital chloridometry,

Beckman instrument), calcium by colorimetric method of Moorehead and Biggs (1974), inorganic phosphorus by the method of Gomori (1941), osmolality by the freezing point osmometer (Advance osmometer model 3).

Packed cell volume was determined by the preparation of the blood in an international microcapillary tube and then centrifuged by microcapillaries centrifuge (Adams micro hematocrit centrifuge, Model 850 Ta), and determined by international microcapillary reader (Hawksley micro hematocrit reader).

Calculation :

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

Pulse pressure = $Ps - Pd$

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

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

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

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

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

Urinary electrolyte excretion = $U_e \times V$

Fractional electrolytes excretion = $U_e \times \frac{V/P \times 100}{GFR}$

Filtered load of electrolytes = $P_e \times GFR$

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

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

Statistical Analysis

Data were expressed as the mean value $\pm$ S.E., the paired t-test was used to estimate the statistical significance of the difference between value obtained form control period and from each experimental period. The unpaired t - test was used to estimate the statistiical significance of the difference between value obtain form intact group and thyroparathyroidectomized group.