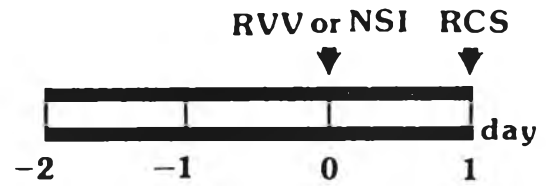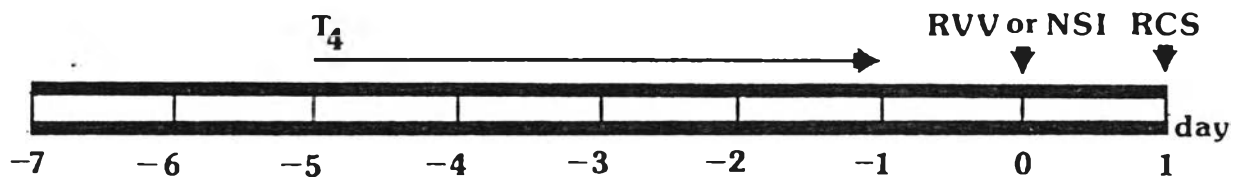# Chapter III

Materials and Methods

Animals and Procedures

The experiments were carried out in male Wistar rats weighing between 250 g to 350 g All animals were housed in individual metabolic cage and had free excess to water and a nutritionally complete diet. The experiment consisted of a pre-experimental period lasting 2 days and was followed by an experimental period.

The following groups were studied. Group I, normal (N) animals (n=8) received normal saline injection. Group II, normal-venom (NV) animals received 1 mg/kg BW of Russell's viper venom subcutaneously. Group III, hyperthyroid (T_4) animals (n=8), animals in this group were induced to be hyperthyroid by received daily subcutaneous injection of exogenous L-thyroxine 10 μg/100 g BW for 5 days and then received normal saline injection. Group IV, hyperthyroid-venom (T_4V) animals (n=8), as in group III, received the same dose of exogenous L-thyroxine for 5 days and then received 1 mg/kg BW of Russell's viper venom. Group V, hypothyroid (TX) animals (n=8), animals in this group were surgically thyroidectomy (to control the level of plasma calcium, calcium gluconate 1 g% was added in drinking water) and received normal saline injection on the 7th day after the surgery. Group VI, hypothyroid-venom (TXV) animals (n=8), as in group

Group N and NV

RVV or NSI   RCS

‑2   ‑1   0   1   day

Group T$_4$ and T$_4$V

T$_4$ →

RVV or NSI   RCS

‑7   ‑6   ‑5   ‑4   ‑3   ‑2   ‑1   0   1   day

Group TX and TXV

TX

RVV or NSI   RCS

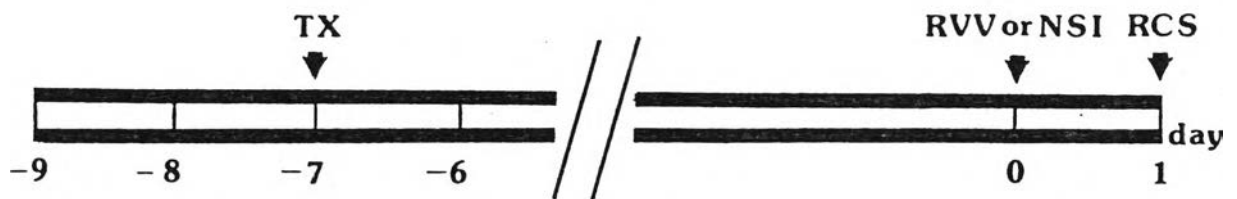‑9   ‑8   ‑7   ‑6   //   0   1   day

Fig.1   Diagrammatic illustration of experimental protocols.

RRV = Russell's viper venom injection

NSI = Normal saline injection

RCS = Renal clearance study

T$_4$ = Exogenous L-thyroxine administration

TX = Thyroidectomy

V, were surgically thyroidectomy and received 1 mg/kg BW of Russell's viper venom on the 7th day after the surgery.

All groups were studied renal clearance 1 day after the venom or normal saline injection. The protocol for this study is shown in Fig.1.

On the day of renal clearance study, the rat was weighed, then anesthetized by intraperitoneal injection of sodium thiobarbiturate (inactin), 10 mg/100 g. BW. Small supplemental doses were given when necessary.

The surgical procedures including a tracheostomy ; common carotid artery, jugular vein and bladder cannulations were carried out. The tracheostomy was performed by using a short piece of PE 240 polyethylene tube, for aspiration of secretions that might block the airway during the course of the experiment. The common carotid artery was cannulated with a PE 90 polyethylene tube for measurement of the arterial blood pressure and for blood sampling. The arterial blood pressure was monitored with a pressure transducer (Statham PE 23 DE) and record on a grass polygraph recorder (Model 79 D). The cannulation of jugular vein was carried out using a PE 90 polyethelene tube for intravenous infusion. The bladder was cannulated with a PE 200 polyethelene tube for urine collection. The rectal temperature was maintained approximately at $37^{\circ}C$ throughout the experiment.

After the surgical procedures had been finished, the solution containing 0.75 g% inulin and 0.12 g% para-aminohippuric acid (PAH) was

administered intravenously via jugular vein at the rate of 0.07 ml/min by infusion pump (Sage Instrument 341 A) to maintain the plasma inulin and PAH concentrations by approximately 20-40 mg% and 2-4 mg% respectively. The rate of infusion was kept constant throughout the course of the experiment.

A period of 45 minutes of infusion was allowed for a stabilization of plasma inulin and PAH concentrations. After the urine flow rate was stable, the urine and blood samples were collected. Urine was collected in pre-weighed urine plastic tube. The duration of urine collection period was between 20 to 40 minutes depending on the urine flow rate. The rate of urine flow was estimated from the change in the weight of the urine in the tube divided by the duration of the urine collection. After the second period of urine collection, an arterial blood sample was collected into the heparinized tube. The blood sample was centrifuged, the hematocrit was then determined and the plasma was frozen for chemical analysis. At the end of the experiment, kidneys were removed and weighed, the tissue was kept with 10% formaldehyde solution for histopathology study.

Determination of blood and urine samples

Inulin concentrations in plasma and urine were determined by the method of Schreiner as described by Smith (1962).

Determination of PAH was carried out according to the method of Bratton and Marshall as modified by Smith (1962).

Routine measurements of sodium and potassium concentrations in plasma and urine were determined by Flame photometer (Beckman, Kina Flame), chloride by chloridometer (Buchler, Digital chloridometer), calcium by the method of cresolphthalein complexone (Moorehead and Biggs,1974), osmolality by using the freezing-point depression method (Osmometer, Model 3D2) the plasma thyroxine (T_4) by ion exchange column chromatography (Berger, and Quinn,1976), the plasma urea and creatinine by 2,3-Butanedione 2-Oxime (Ritcher and Lapointe,1962) and Alkaline Picrate (Smith, 1962) respectively.

Hematocrit was determined by the preparation of blood in an international microcapillary tube then centrifuged by Cray Adams micro hematocrit centrifuged (Model 850 Ta) and measure by Hawksley micro hematocrit reader.

Calculations

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

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

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

$$\text{Osmolar clearance} = C_{Osm} = U_{Osm} \cdot V / P_{Osm}$$

$$\text{Free water clearance} = C_{H_2O} = V - C_{Osm}$$

$$\text{Urinary excretion of electrolytes} = U_e \cdot V$$

Fractional excretion of electrolytes(FE_e) $=$ $U_e.Vx1oo/(P_exGFR)$

Effective renal blood flow (ERBF) $=$ ERPF/(1-Hct)

Filtration fraction (FF) $=$ GFRx100/ERPF

Renal vascular resistance (RVR) $=$ MABPx1333x60/(ERBFx1000)

Statistical Analysis

Data was processed according to the unpaired t-test. p-value less than 0.05 was considered significantly.