# CHAPTER III

# MATERIALS AND METHODS

## Animal preparation

Experiments were carried out in twenty adults male dogs, weighting 8-16 kgs. The animals were fasted for 12 hours. preceding the operation. On the day of the experiment, the dog was anesthetized with the intravenous injection of sodium pentobarbital 25 mg/kg.bw initially, and recieved subsequent doses of 1-2 mg/kg.bw when necessary to maintain light anesthesia throughout the experiment.

## Surgical and Experimental procedure.

A tracheal cannula was inserted by tracheostomy to secure free airways. A jugular vein was cannulated with polythylene tube (PE 180) for infusion of inulin and PAH. In order to study renal clearance, the priming solution containing p-aminohippurate (PAH) 1.2% and inulin 5% in isotonic saline were administered 0.5 ml/kg/bw then the sustaining solution composed of 0.12% and 0.75% of PAH and inulin respectively, were infused at the rate of 1.8 ml/min with peristaltic pump (Eyla Model 3).

One of femoral artery was cannulated with polyethylene tube (PE 200) for blood pressure recordings and collection of blood sample. A pressure transducer (PE 23 AA) and polygraph (Grass Model 7) recorder were used for blood pressure and heart rate estimation. Red rubber catheter was cannulated into left renal vein via femoral vein for collection of renal venous blood sample.

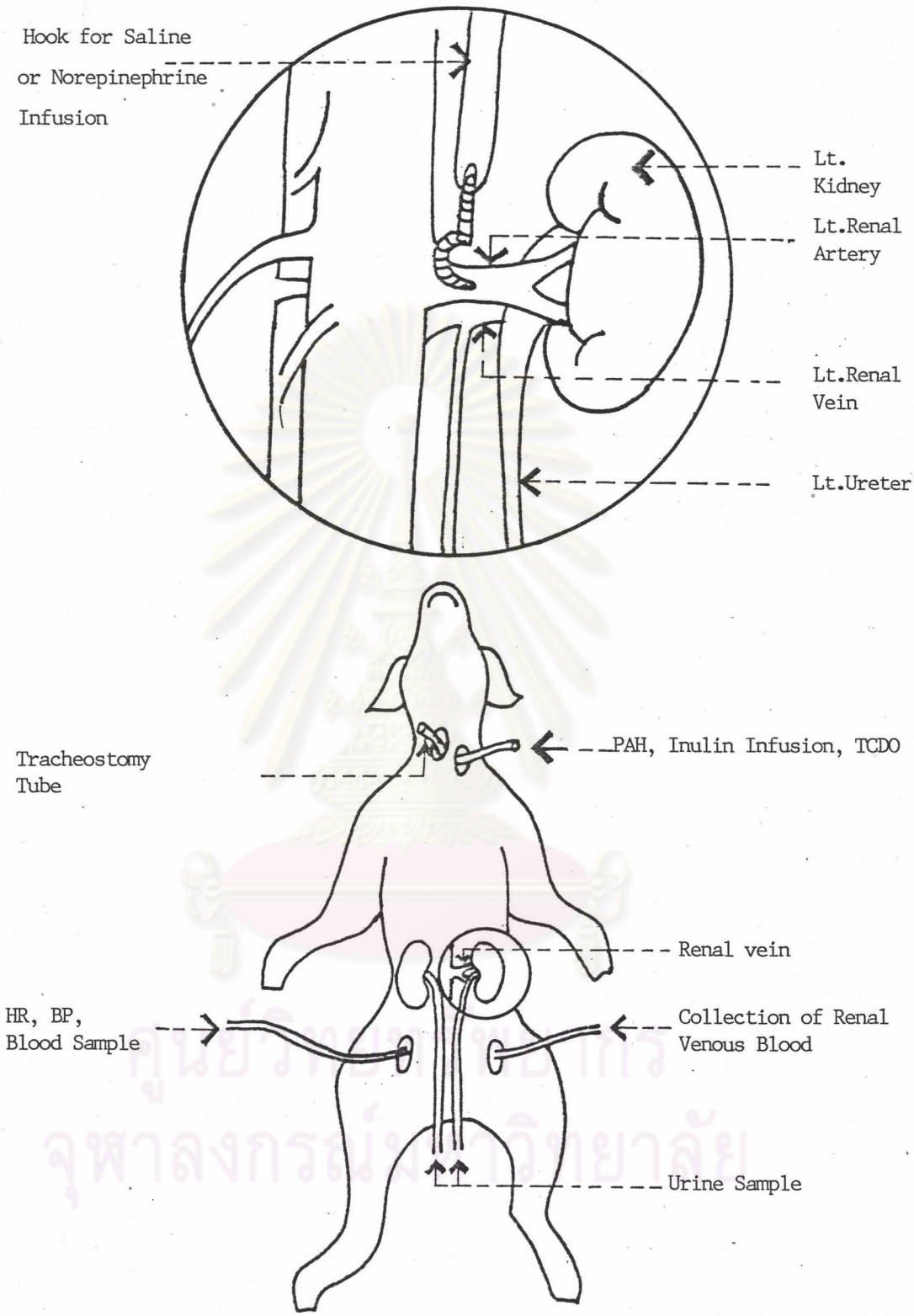


Fig. D. : A diagrammatic illustration of the technique used for studying renal hemadynamics and renal functions.

Bilateral flank incision were made, both ureters were catheterized via vetroperitoneal approach with polyvinyl catheter (PV 190) for urine collection. A hook shape 23 gauge needle atached to polyethelene catheter (PE 50) was hooked at the base of left renal artery, anterograde to renal arterial flow, for infusion of 0.9% NSS and dissolved norepinephrine 0.75 ug/kg.bw/min during control and experimental periods respectively (Fig. D)

After an hour of infusion and the rate of urine flow was steady, duplicated sample for clearance study were obtained. Two urine sample were collected during 20 mins interval in control and NE infusion period and during 30 mins in post NE infusion period. One arterial blood sample and renal venous blood sample was drawn at the midpoint of the urine collection.

Experimental protocols

To study the effects of TCDO in NE-induced ARF, sixteen dogs were divided into four groups.

Group I Four dogs were used as control animals. A minimum of 60 minutes was allowed for equilibration and stabilization. The systemic blood pressure and heart rate were recorded. Prior to NE infusion 0.9% NSS was infused into renal artery by the rate 1 ml/min with syringe pump (Sage Instruments Model 341 A). After that blood and urine sampled for the control(0) period. The dose of 0.75 ug/kg.bw of NE, dissolved in 0.9% NSS 40 ml, was infused intrarenal arterially with syringe pump at the rate of 1 ml/min. The post NE infusion period of 1 1/2, 2, 2 1/2, 3 and 4 hour were sampled consecutively till the end of experiment. The arterial, renal venous blood gas and packed cell volume were measured closely to the end of each sampling period.

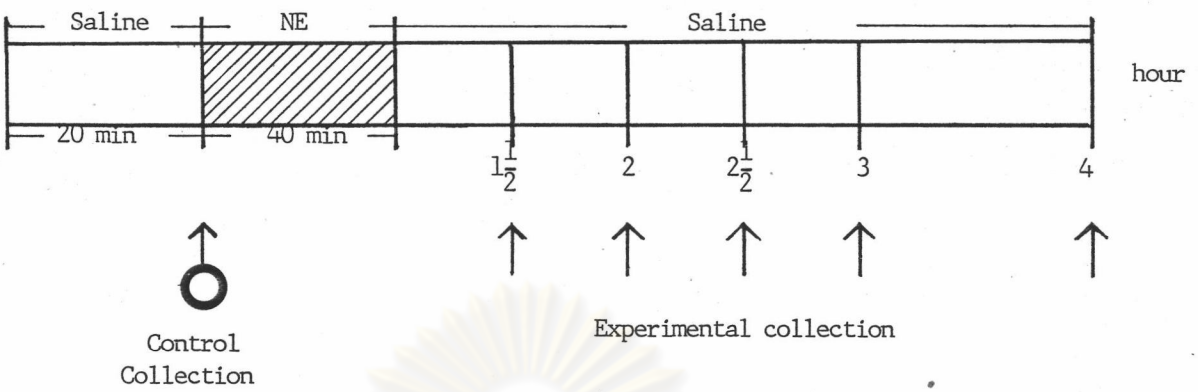


Fig. E Diagrammatic illustration of experimental protocol of group I

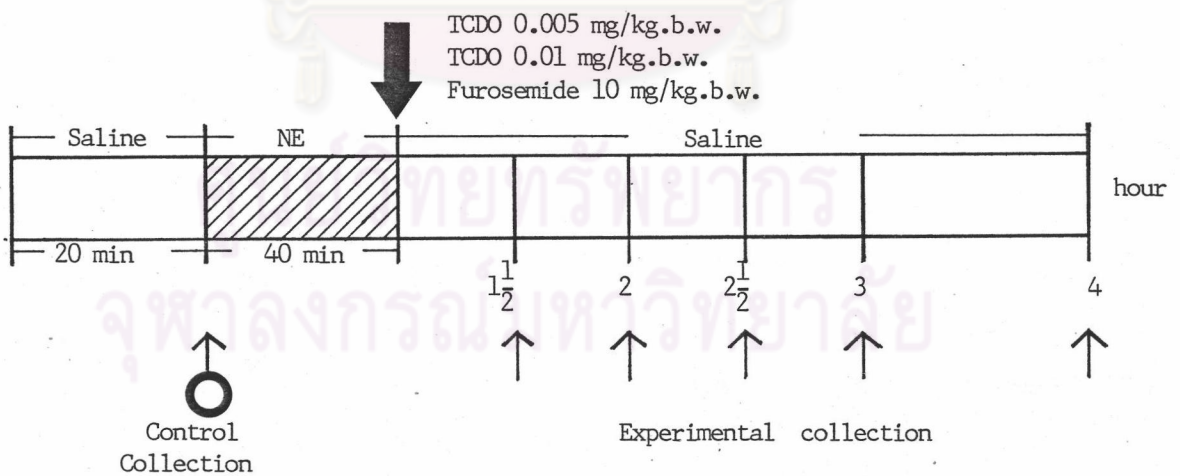


Fig.F Diagrammatic illustration of experimental protocol of group II, III and IV.

Group II Four dogs were treated in the same manner of group I but after 40 mins of NE-infusion, 0.005 mg/kg.bw of TCDO was immediatly infused via jugular vein.

Group III Four dogs were treated in the same manner of group I but after 40 mins of NE infusion, 0.01 mg/kg/bw. of TCDO was immediatly infused via jugular vein.

Group IV Four dog were treated in the same manner of group I but after 40 mins of NE infusion, 10 mg/kg.bw. of Furosemide was immediatly infused via jugular vein.

**Determination of blood and urine samples**

Determiantion of plasma and urine PAH concentrations were carried out by the method of Bratton and Marshall as modified by Smith (1962). Plasma and urinary inulin concentrations were determined by the anthrone method as described by Davidson *et al* (1963). Using the Fick's principle, PAH clearance was used determination of effective arenal plasma flow (ERPF) and inulin clarance 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, inorganic phosphorus by the method of Gomori (1941), osmolality by the freezing point osmometer (Advance osmometer model 3).

Arterial and renal venous blood gas were determined by Blood Gas Analyzer (model 165/2), packed cell volume was determined by the preparation of 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 = $Pd + 1/3 (Ps - Pd)$

Glomerular filtration rate (GFR) = $\frac{Uin\ V}{Pin}$

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

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

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

Urinary electrolytes exretion = $U_E\ V$

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

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

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

Renal oxygen uptake = $(A_o - V_o) \times ERPF$

Renal respiratory quotient (R.Q.) = $\frac{CO_2\ Production \times RPF}{O_2\ Consume}$

Oxygen extraction ratio = $\frac{A_o - V_o}{A_o}$

Statistical Analysis

Data were expressed as the mean value ± S.D., the paired t-test was used to estimate the statistical significance of the differance between value obtained from control period and from each experimental period. The unpaired t-test was used to estimate the statistical significance of the difference between value obtained from control group and treatmanted group.