Chapter III

Material and Methods

Animals preparation

Adult male mongrel dogs, weighing 10-18 kgs. were used in the experiments. The animals were fasted for 12 hours preceding the investigation. On the day of the experiment, the dog was anesthetized with pentobarbital sodium 25 mg/kg.bw. intravenously. Supplemental doses of pentobarbital were administered as required during the study to maintain an even state of anesthesia. A tracheal cannula was inserted to secure free airway. Two femoral veins were cannulated with polyethylene tubes (PE 180). Before the clearance study, the priming solution containing P-aminohippurate (PAH) 1.2 gm% and inulin 5 gm% in isotonic saline (adjust to pH 7.4) was administered intravenously 0.5 ml/kg.bw. and followed immediately by sustaining solution composed of 0.12 gm% and 0.5 gm% of PAH and inulin respectively, were infused at the rate of 1.4 ml/min. The rate of infusion was kept constant throughout the course of experiment by a peristaltic pump (Eyla Model 3). One of femoral artery was connulated with polyethylene tube (PE 200) for blood collection and connected to the pressure transducer (PE 23 AA) for recording blood pressure and heart rate by polygraph (Grass model 7). Both ureters were reached by bilateral flank incisions with a retroperitoneal approach and tubulated with polyvinyl catheter (PV 190) for urine collection.

After an hour of infusion of inulin and PAH solution, and the rate of urine flow stabilized, urine samples were obtained and the blood sample was collected at the midpoint of the urine collection.

Experimental protocols

Fifteen dogs were devided into three groups. (fig. 3.2)

Group 1 : Effects of omeprazole on renal acid excretion in the hypokalemic dogs induced by furosemide infusion.

Five dogs were used in this group. The animal was induced to be a hypokalemic animal by using furosemide infusion (Alguire et al., 1974). After 1 hour of infusion of sustaining inulin and PAH solution, the control sample of urine and arterial blood were obtained. The animal was given intravenously with bolus dose of furosemide 1 mg/kg. bw. and followed immediately by continuous dose of furosemide dissolved in 0.9 % isotonic saline at the rate of 0.3 mg/kg.bw./hr. throughout the experiment. Then the animal was injected intravenously with polyethylene-glycol (PEG # 400) 10 ml alone prior to omeprazole. And followed by 5 μmole/kg.bw. of omeprazole, dissolved in 10 ml of PEG # 400, was injected intravenously. Cardiovascular and renal functions were measured at 15, 30, 60, 90, 120, 150, 165, 180, 195, 210, 225 and 240 minutes.

Group 2 : Effects of omeprazole on renal acid excretion in the hypokalemic dogs induced by prolonged chronic oral furosemide administration

Five dogs were used in this group. The animal was induced to be a hypokalemic animal by using prolonged oral furosemide administra-

tion. (Bosch et al., 1977). Before-experimental period, the animal was given with oral furosemide in the dose of 80 mg/day for 3 days. On the day experiment, the animal was treated in the same manner of group 1. Cardiovascular and renal functions were measured at 15, 30, 60, 90, 120, 150, 165, 180, 195, 210, 225 and 240 minutes.

Group 3 : Effects of omeprazole on renal acid excretion in the hypokalemic dogs induced by insulin infusion

Five dogs were treated in the same manner of group 1. But insulin was used instead of furosemide to induce hypokalemia, which was infused intravenously with the bolus dose of insulin 106 mū/kg. bw. and followed immediately by sustaining dose of insulin in 0.9% isotonic saline in the rate of 6 mū/kg.bw./min. throughout the experiment (Rossetti et al., 1987). In this group, glucose was infused after insulin infusion to prevent hypoglycemia. By the maintainance of normoglycemia, the release of epinephrine could avoid. Cardiovascular and renal functions were measured at 15, 30, 60, 90, 120, 150, 165, 180, 195, 210, 225, 240 minutes.

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).

The compositions in the plasma and urine were determined as followed; sodium and potassium flame photometer (KliNa flame operating,

Beckman instrument), chloride by Chloride/Carbon dioxide analyzer (Beckman instrument).

Determination of Urinary Titratable Acid and Ammonium (Folin method)

Titratable acidity and ammonium were estimated by titrating a fresh specimen of urine with 0.05 NNaOH, using 1% phenolphthalein as an indicator, the end point pH is 8.3. Transfer 1 ml of urine to a test tube and add 0.2 gm of powdered potassium oxalate ($K(COO)_2 H_2O$) to precipitate the calcium 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 0.05 NNaOH 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 titrations is the volume of NaOH used. To the above titrated mixture, add 2 ml neutral formalin solution and mix well. Titrate again with 0.05 NNaOH until first permanent pink. The urinary titratable acid excretion and ammonium excretion were calculated as following :

$$\text{Normality of ammonium or titratable acid} = \frac{\text{0.01 N NaOH x ml of 0.01 N NaOH used}}{\text{ml of urine (1 ml)}}$$

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

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

Calculation :

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

Pulse pressure (PP) = $Ps - Pd$

Glomerular filtration rate (GFR) = $\frac{Uin \times V}{P_{in}}$

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

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

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

Urinary electrolyte excretion (U_eV) = $U_e \times V$

Fractional electrolyte excretion (FE_e) = $\frac{U_eV/P_e \times 100}{GFR}$

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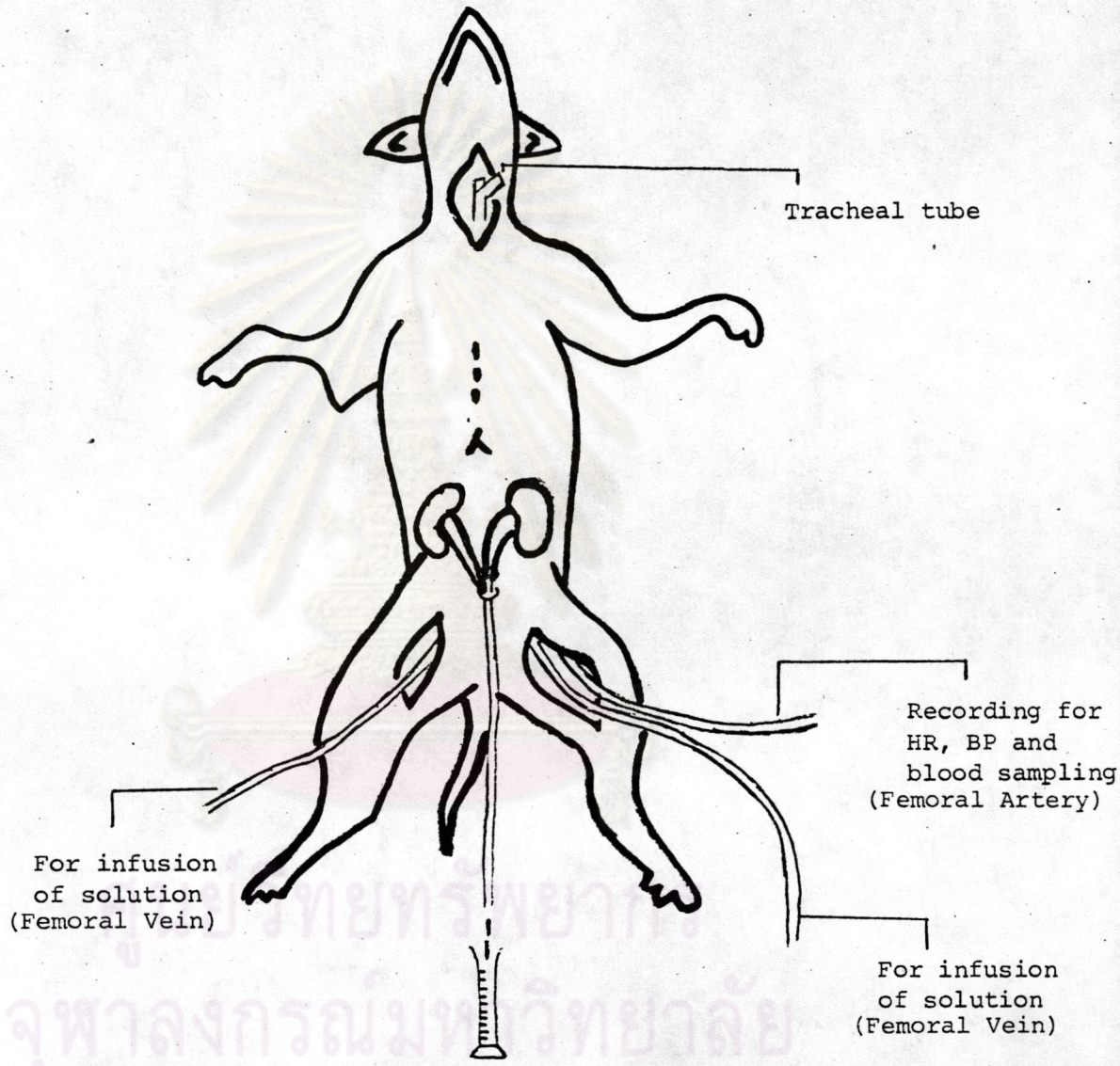
Fig. 3.1 : Scheme of experiment



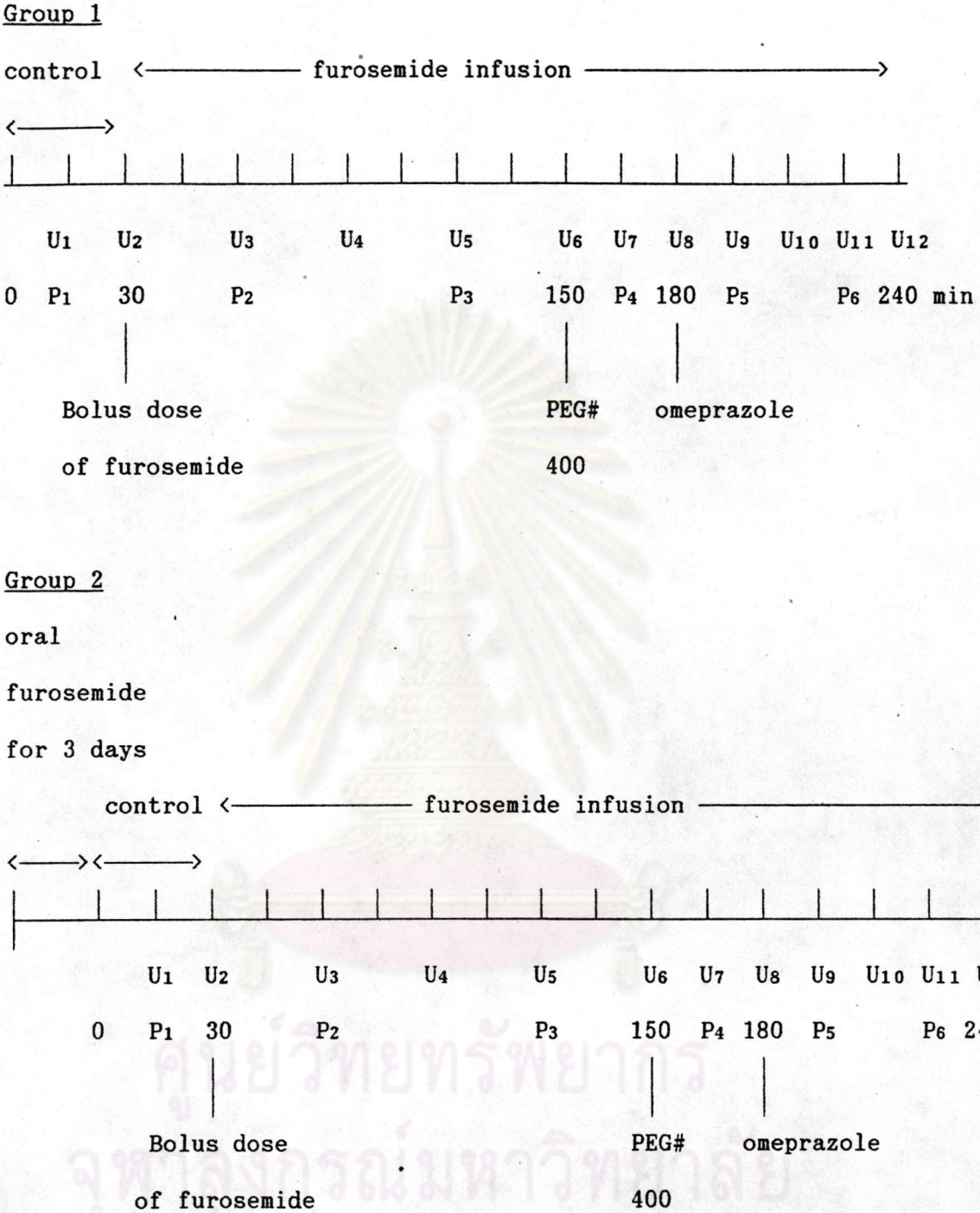


Fig. 3.2 : Diagramatic illustation of experimental protocols

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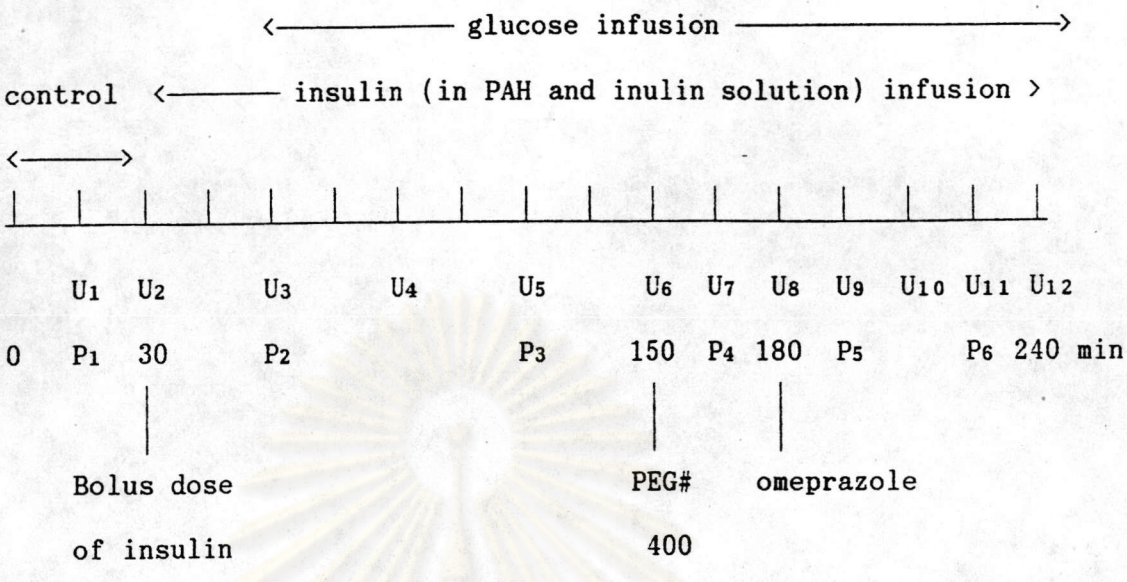
Group 3



U = Urine samples were collected

P = Blood samples were collected

Fig. 3.3 : Diagramatic illustation of experimental protocols

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Statistical analysis

Data were reported as the mean value ± S.D.. The paired t-test was used to estimate the statistical significance of difference between value obtained from the control period and from each period of the experiment.

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