Materials and Methods

Experiments were conducted on adult male mongrel dogs, weighed 10-15 kg. Two series of experiments were carried out; the first (series I) to investigate the minimal lethal dose of the Russell's viper venom in dogs; the second (series II) to study the effects of the Russell's viper venom on cardio-vascular and renal functions.

Series I

The first series of experiments were performed on 14 conscious male mongrel dogs, weighing between 10-15 kg. Animals were fasted for 12 hours before the experiment. On the day of experiments, polyethylene catheter (PE 180) was inserted into a jugular vein for venom injection and blood sample collections. The Russell's viper venom was prepared by dissolution the lyophilized venom 1 mg. in 1 ml. of 0.9 % sodium chloride. Before venom injection, control blood was collected via jugular vein. Each dog was injected a single dose of the venom, which was varied from 0.20, 0.25, 0.35, 0.40 and 0.50 mg./kg.bw. After envenomation, blood samples were collected again at 15 minutes, 1 hour, 3 hours, 24 hours and 48 hours respectively. Blood samples were determined for haemoglobin (Hb) and packed cell volume (PCV). Measurement for rectal temperature, heart rate and respiratory rate were made with the observation of

clinical signs on each period after envenomation. These data were determined for further studies in the second series.

Number of the dogs injected different doses of venom are as followed:

number of dogs	doses of the venom (mg./kg.bw.)
2	0.20
4	0.25
4	0.35
2	0.40
2	0.50

Series II

The second series of the experiments were performed to study the effects of Russell's viper venom on the cardio-vascular and renal functions. Eight male mongrel dogs weighing between 10-15 kg. were fasted for 12 hours before the experiment. They were anaesthetized with thiopentone sodium 20-25 mg./kg.bw. intravenously and supplemental doses were given as required to maintain anaesthesia. One of femoral arteries was cannulated with polyethylene tube (PE 200) for arterial blood sample collection, and connected to the pressure transducer (P23AA) for recording blood pressure and heart rate on a Grass polygraph (Grass model 7). The femoral vein was cannulated with polyethylene tube (PE 180) for venom injection, and continual sustaining inulin and PAH infusion (Eyela micro-infusion pump model MP-3), and for injection of Evans blue (T-1824). The polyethylene catheter was introduced via urethra

into the bladder for urine collection. Before the clearance study, the bladder was empty and urine sample would be collected when the rate of urine flow was constant.

Before the clearance study, the priming solution containing 200 mg. of p-aminohippuric acid (PAH) and 400 mg. of inulin in 8 ml. of isotonic saline (adjusted to pH 7.4) was injected intravenously into femoral vein and followed immediately by the sustaining solution at the rate of 1.6 ml./min. The rate of infusion was kept constant throughout the course of the experiment in order to maintain the plasma concentration of inulin 0.20 mg./ml. and PAH 0.03 mg./ml. The composition of the sustaining solution is PAH 4 mg. and inulin 8 mg./ml. of 0.9 % sodium chloride (pH being adjusted to 7.4).

After starting the infusion of inulin and PAH, a period of $\frac{1}{2}$-1 hour was allowed for stabilization. The initial blood and urine samples were taken. An arterial blood sample was collected from the femoral artery at the midpoint of each 10-minutes urine collection. Blood and urine samples were determined for inulin, PAH, osmolality, sodium, potassium, chloride, calcium and phosphorus. Blood sample was also measured for the haemoglobin and packed cell volume.

According to the experiment in the first series, it was found that the minimal lethal dose should be in the range of 0.20-0.35 mg./kg.bw. But when these doses were injected in unconcious dogs in the second series of the experiment, it was found that the venom and anaesthetic drug were synergistic.

Therefore, the dogs were such more depressed and died easily. According to the purpose of the experiment, the dose had to be adjusted to 0.10 mg./kg.bw., which was practical to the second series of the experiment.
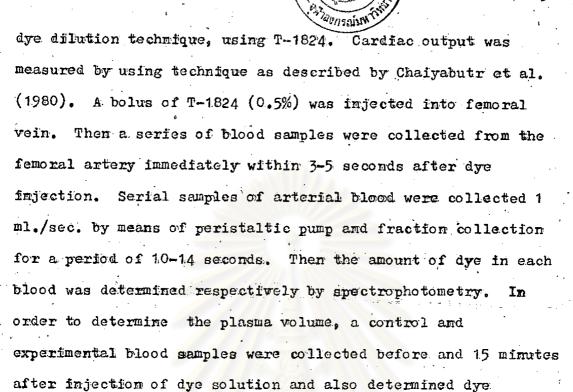
After the control period, lyophilized venom 0.10 mg./kg.bw. was dissolved with 0.9 % isotonic saline solution 20 ml. was injected intravenously into the femoral vein. After the injection, the experimental periods were carried out as in the control period at 2, 24 and 48 hours after envenomation respectively.

Determination of transport of PAH

After the clearance study, the priming solution containg PAH 50 mg. in 2 ml. of isotonic saline (adjusted to pH 7.4) was injected into femoral vein and followed immediately by the sustaining solution of PAH which contained PAH 200 mg. in 20 ml. of isotonic saline (adjusted to pH 7.4) at the rate of 1.6 ml./min. for 20 minutes. According to the clearance study, the plasma concentration of PAH had been raised to a level of 0.03 mg./ml. already. Therefore, the level of plasma PAH concentration was raised to the higher level of 0.09 mg./ml. Then blood and urine collections were taken periodically in the same way as the former description. These samples were determined for transport of PAH.

Determination of cardiac output and plasma volume

Both cardiac output and plasma volume were measured by

dye dilution technique, using T-1824. Cardiac output was measured by using technique as described by Chaiyabutr et al. (1980). A bolus of T-1824 (0.5%) was injected into femoral vein. Then a series of blood samples were collected from the femoral artery immediately within 3-5 seconds after dye injection. Serial samples of arterial blood were collected 1 ml./sec. by means of peristaltic pump and fraction collection for a period of 10-14 seconds. Then the amount of dye in each blood was determined respectively by spectrophotometry. In order to determine the plasma volume, a control and experimental blood samples were collected before and 15 minutes after injection of dye solution and also determined dye concentration by spectrophotometry.

The methods of determination blood and urine samples

PAH was determined by the method of Bratton and Marshall as modified by Smith (1962). Determination of inulin was carried out by the method of Schreiner as described by Smith (1962).

Routine measurement of sodium and potassium concentrations in plasma and urine were determined by flame photometry (Klina flame operating; Beckman instrument), chloride by chloridometer (Buchler digital chloridometer; Beckman instrument), calcium by atomic absorption (Varian Tectron model AA 775), phosphorus by the method of Gomori (1941), osmolality by using the freezing-point depression method (Advance osmometer model 3).

010578

Cardiac output was determined by Evans blue dilution technique and was calculated as described by hamilton et al. (1948). The plasma volume was calculated by the method of Kolmer (1951). Packed cell volume was determined by the centrifuge, and measured with an international microcapillary reader. Haemoglobin was measured by the spectrophotometric method of Drabkin and Austin (1935).

Calculations

The following symbols are used throughout the calculation:

V = the rate of urine flow(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.)

Pt_{PAH} = plasma concentration of PAH when loaded PAH (ug./ml.)

Ut_{PAH} = urine concentration of PAH when loaded PAH (ug./ml.)

$T. PAH$ = transport of PAH (ug./min.)

P_{Osm} = plasma osmolality (mOsm./l.)

U_{Osm} = urine osmolality (mOsm./l.)

C_{Osm} = osmolar clearance (ml./min.)

C_{H_2O} = free water clearance (ml./min.)

P_e = plasma concentration of electrolytes (mEq./l.)

U_e = urine concentration of electrolytes (mEq./l.)

Hb   = haemoglobin (mg.%)

PCV   = packed cell volume (%)

MCHC   = mean corpuscular haemoglobin concentration (mg.%)

MABP   = mean arterial blood pressure (mm.Hg.)

TPR   = total peripheral resistance (dynes-sec./cm^5.)

RVR   = renal vascular resistance (dynes-sec./cm^5.)

Using the Fick's principle, PAH clearance was measured for determination of effective renal plasma flow (ERPF), and inulin clearance for glomerular filtration rate (GFR).

The following calculations were performed:

glomerular filtration rate (GFR) $= \dfrac{U_{In} \times V}{P_{In}}$

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

effective renal blood flow (ERBF) $= \dfrac{ERPF}{(100-PCV)}$

filtration fraction $= \dfrac{GFR}{ERPF}$

transport of PAH $= (Ut_{PAH} \times V) - (GFR \times Pt_{PAH})$

osmolar clearance $= \dfrac{U_{Osm} \times V}{P_{Osm}}$

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

filtered load of electrolytes $= GFR \times P_e$

urinary electrolyte excretion $= U_e \times V$

fractional excretion $= \dfrac{U_e \times V}{P_e \times GFR}$

renal fraction $\qquad = \dfrac{\text{ERBF} \times 100}{\text{cardiac output}}$

total peripheral resistance (TPR) $\quad = \dfrac{\text{MABP} \times 1333 \times 60}{\text{cardiac output} \times 1000}$

renal vascular resistance (RVR) $\quad = \dfrac{\text{MABP} \times 1333 \times 60}{\text{ERBF} \times 1000}$

mean corpuscular haemoglobin concentration (MCHC)

$\qquad\qquad\qquad\qquad = \dfrac{\text{Hb} \times 100}{\text{PCV}}$

Statistics

The paired t-test was used to estimate the statistical significance of difference between values obtained from the control period and from each period of the experiment.