CHAPTER IV

Results

Series I

The investigation of minimal lethal dose (MLD)

In the first series of the experiment, the rate of survival of the dogs injected different doses of Russell's viper is shown in the table 1. It is found that dogs injected at the dose of 0.40 and 0.50 mg./kg.bw. died 100 %, whereas at the dose of 0.20, 0.25 and 0.35 mg./kg.bw. died only 50 %. Therefore, the minimal lethal dose is in the range of 0.20-0.35 mg./kg.bw.

After venom injection, some dogs had nystagmus during the initial period. There was contraction of the abdominal muscle immediately, followed by defication and urination. Then the dog had vomiting and salivation. Delayed blood clot also started at 30 minutes after envenomation.

After half an hour, the dog showed signs of depression and hyperthermia. There was usually bloody discharge from gum, anus and incision wound on the skin due to defect of blood clot. At this period, it was found that packed cell volume and haemoglobin increased, whereas heart rate and respiratory rate were irregular. The clinical signs during the first hour after envenomation was shown in table 2.

Table 1. Survival rate of the dogs after injection of different doses of Russell's viper venom.

Doses of the venom (mg./kg.bw.)	Numbers of the dogs	Numbers of the survivals	Numbers of the deaths	Rate of survivals (%)
0.20	2	1	1	50
0.25	4	2	2	50
0.35	4	2	2	50
0.40	2	0	2	0
0.50	2	0	2	0

Table 2. The clinical signs of the dogs after injection of Russell 's viper venom.

time after injection	clinical signs
0 - 30 minutes	Abdominal muscle contraction
	Defication
	Urination
	Vomiting and salivation
	Nystagmus (0-10 minutes)
	Delayed blood clot
30 - 60 minutes	Depression
	Hyperthermia
	Bloody discharge
	Irregular heart rate
	Irregular respiratory rate
	Increased packed cell volume
	Increased haemoglobin

Series II

Changes in cardiovascular function

In the first 10 minutes after envenomation, both systolic and diastolic blood pressure decreased significantly ($P < 0.001$), as shown in the figure 1. Mean arterial blood pressure and pulse pressure decreased significantly from 133 $\pm$ 22 to 74 $\pm$ 22 mm.Hg. and from 47 $\pm$ 11 to 25 $\pm$ 8 mm.Hg. (mean $\pm$ S.D.) respectively ($P < 0.01$). After a period of 10 minutes, mean arterial blood pressure started to increase gradually, until 1-2 hours they returned to the control level at 138 $\pm$ 22 mm.Hg. At 24 hours after envenomation, systolic blood pressure decreased slightly, and little increased at 48 hours after envenomation. Diastolic blood pressure decreased significantly to 88 $\pm$ 16 mm.Hg. ($P < 0.05$) at 24 hours. It gradually increased again at 48 hours, however, it was still lower than the control level. These changes of systolic and diastolic blood pressures caused the significant increase in pulse pressure during 24 and 48 hours after envenomation (figure 1). Whereas mean arterial blood pressure decreased to 110 $\pm$ 15 mm. Hg. in 24 hours but increased back to 127 $\pm$ 17 mm.Hg. in 48 hours.

As shown in the figure 2 and table 3, heart rate decreased significantly from 157 $\pm$ 37 to 128 $\pm$ 31 beat/min. ($P < 0.05$) in the first 10 minutes after venom injection. However, it gradually increased and returned to the control level within 2 hours and no further change till the end of the

Table 3 Effect of Russell's viper venom on cardiovascular parameters in dogs. (Mean ± S.D. for 8 dogs)

	control	2 hours	24 hours	48 hours
M.A.B.P. (mm.Hg.)	133.33 ± 22.06	138.96 ± 22.57	110.00 ± 15.33	127.08 ± 17.20
P.P. (mm.Hg.)	47.50 ± 11.95	43.75 ± 11.26	63.75* ± 11.26	73.75*** ± 14.08
H.R. (beat/min.)	167 ± 37	166 ± 31	153 ± 27	159 ± 34
C.O. (lit/min.)	1.57 ± 0.29	1.55 ± 0.30	1.79 ± 0.51	1.82 ± 0.48
S.V. (ml./beat)	9.52 ± 1.23	9.62 ± 2.20	11.76* ± 3.19	11.42* ± 1.32
P.V. (lit.)	0.75 ± 0.12	0.73 ± 0.08	0.76 ± 0.26	0.75 ± 0.19
B.V. (lit.)	1.14 ± 0.18	1.16 ± 0.15	1.01 ± 0.32	0.99 ± 0.28
T.P.R. (10^3 dyne-sec./$cm.^5$)	7.104 ± 2.328	7.445 ± 2.180	5.249* ± 1.524	5.830 ± 1.195
P.C.V. (%)	33.25 ± 4.43	38.75** ± 5.50	28.00 ± 9.47	25.25*** ± 6.20
Hb (mg.%)	11.18 ± 3.02	12.02 ± 3.52	9.17* ± 2.97	7.81** ± 1.71
M.C.H.C. (mg.%)	35.65 ± 6.70	32.11 ± 6.09	35.26 ± 6.58	34.27 ± 8.23

Values were statistically significantly different from the control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

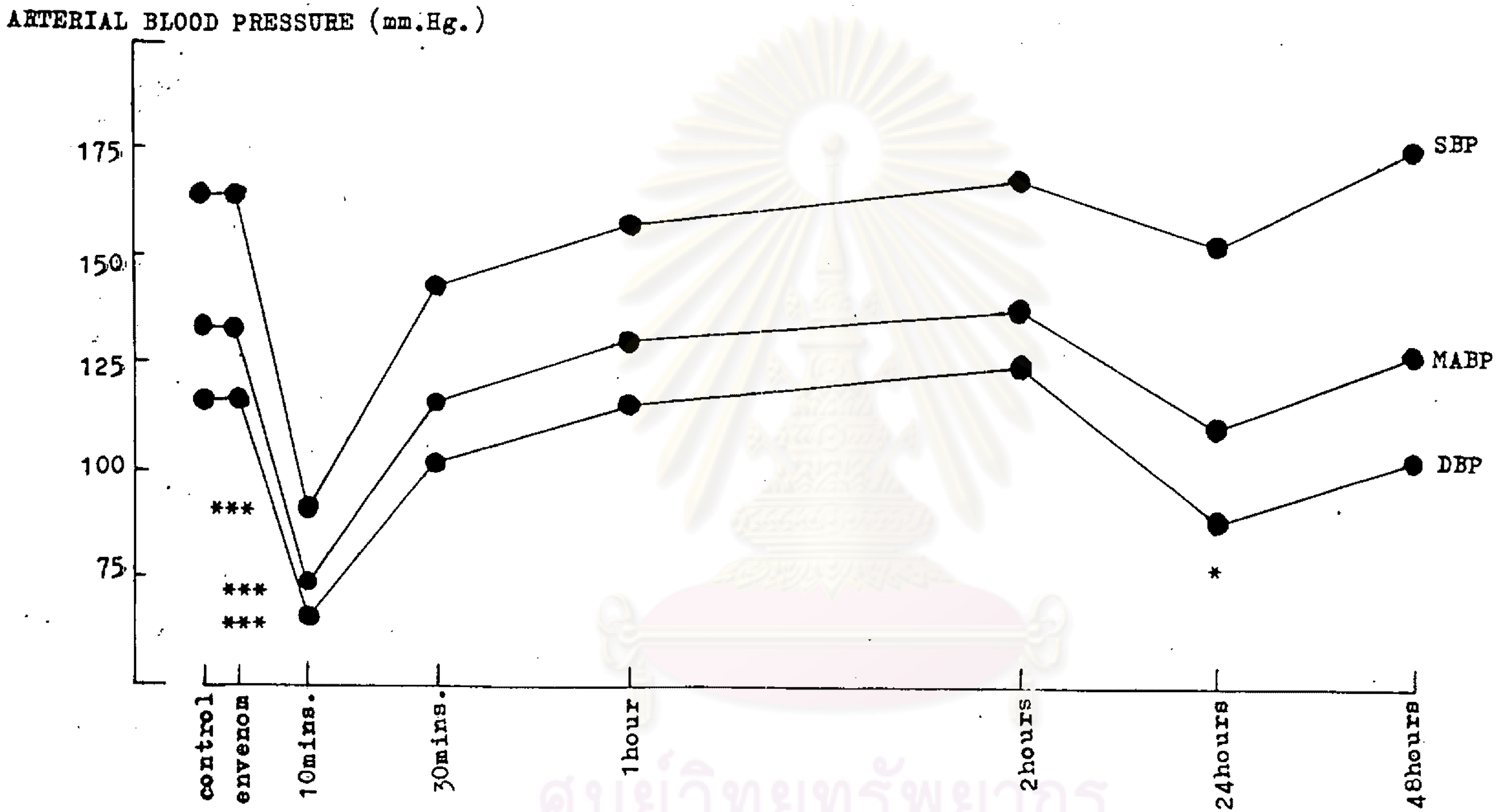
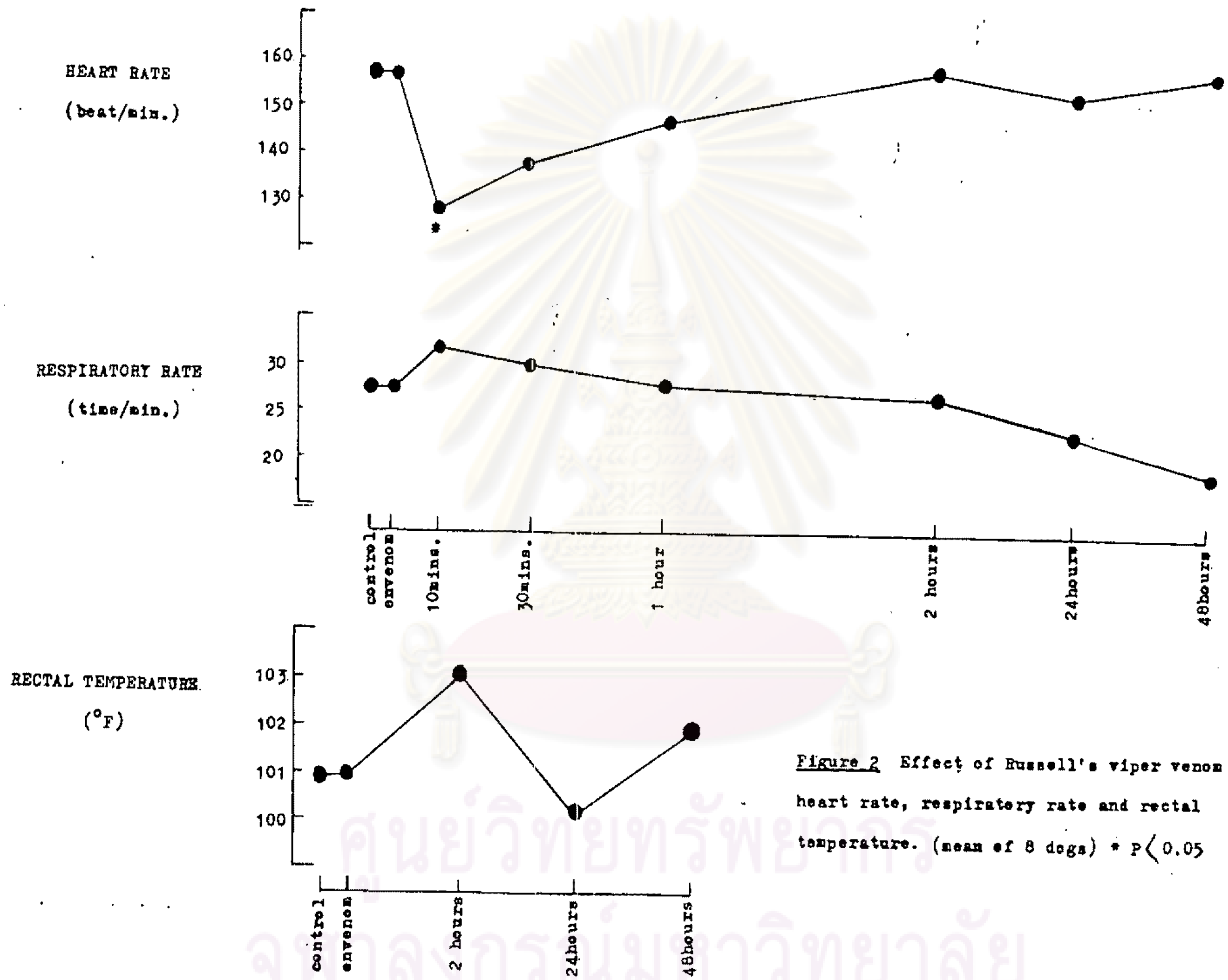
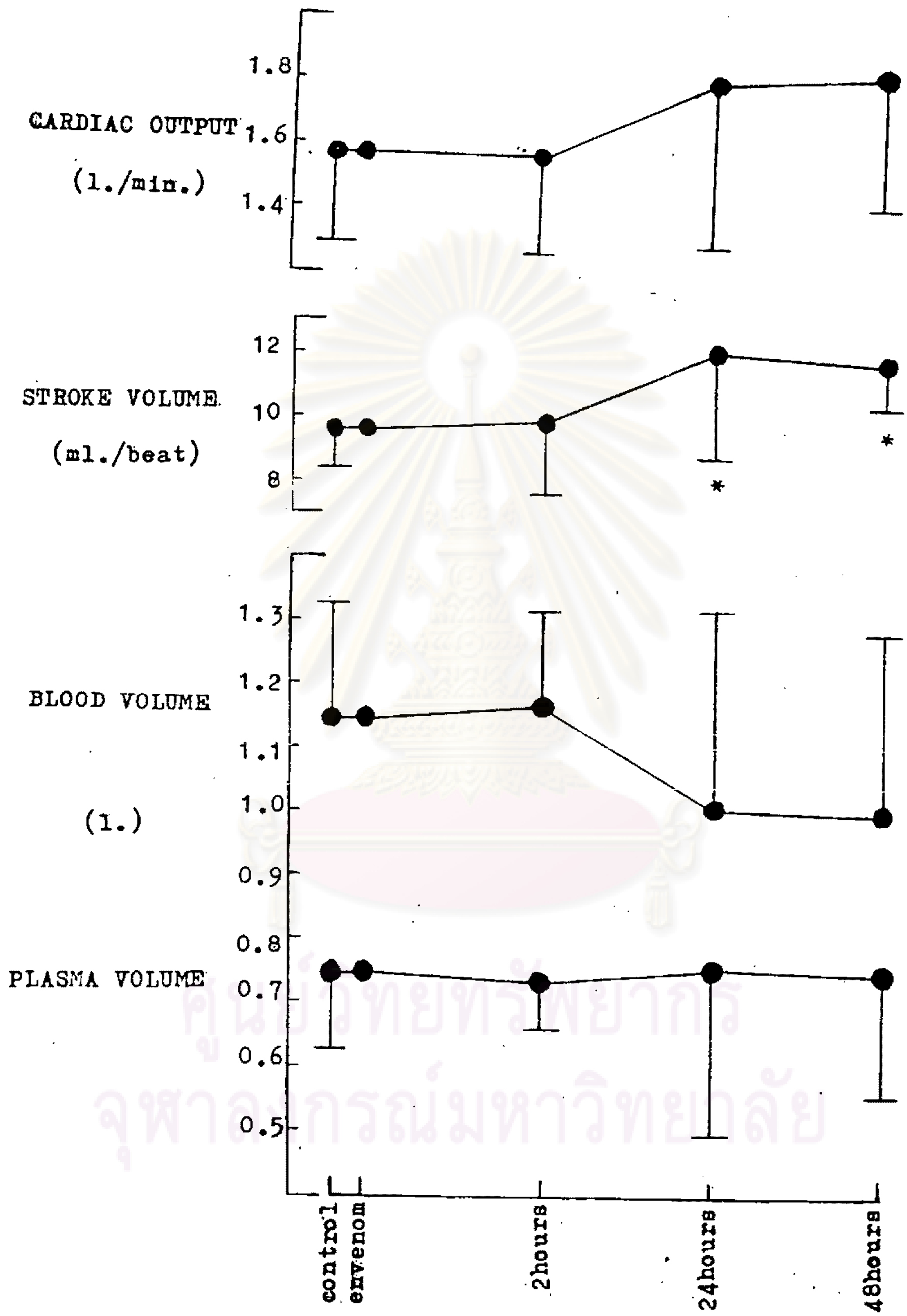


Figure 1 Effect of Russell's viper venom on arterial blood pressure. (mean of 8 dogs)

MABP = Mean Arterial Blood Pressure, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, *** P$<$0.001, ** P$<$0.01, * P$<$0.05



<u>Figure 2</u> Effect of Russell's viper venom on heart rate, respiratory rate and rectal temperature. (mean of 8 dogs) * $P < 0.05$



<u>Figure 3</u> Effect of Russell's viper venom on cardiac output, stroke volume, blood volume and plasma volume. (Mean and S.D. of 8 dogs) * $P<0.05$

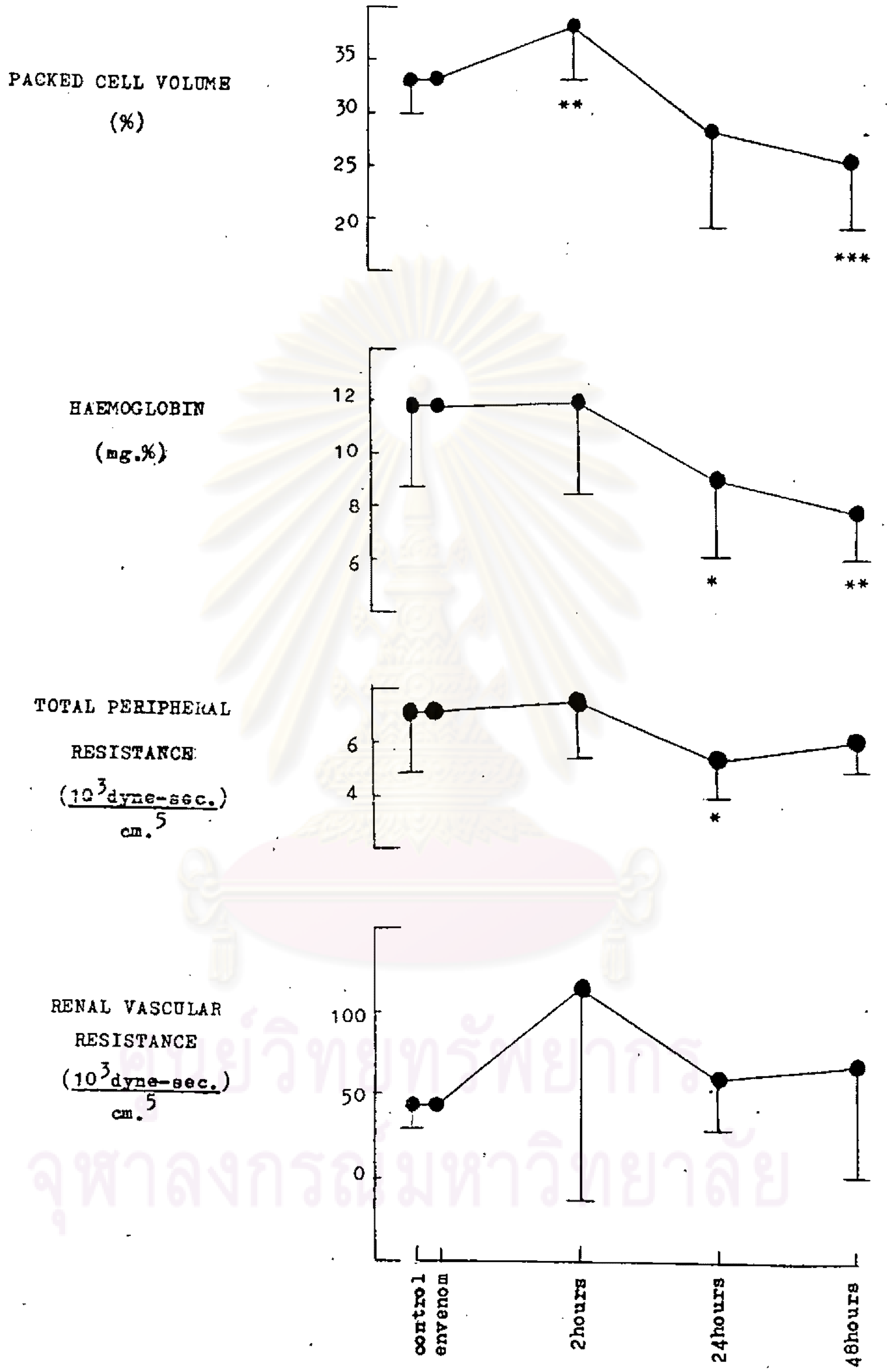


Figure 4 Effect of Russell's viper venom on packed cell volume, haemoglobin, total peripheral resistance and renal vascular resistance. (Mean and S.D. of 8 dogs) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

experiment. Cardiac output showed a tendency to fall in the first 2 hours after venom injection, but statistically insignificant. At 24 and 48 hours after envenomation, cardiac output increased slightly as compared with the control period whereas stroke volume increased significantly during the second day. No significant change in plasma volume was shown during the period of the experiment (figure 3).

The packed cell volume increased significantly from 33 $\pm$ 4 to 38 $\pm$ 5 % at 2 hours after envenomation. At 24 hours, it decreased lower than the control level and still decreased significantly to 25 $\pm$ 6 % ($P < 0.001$) at the end of the experiment. Haemoglobin increased slightly by approximately 2 % at the first period of the experiment, then decreased significantly at 24 and 48 hours of the experimental period (figure 4).

As shown in the figure 4, the total peripheral resistance increased at the first period of the experiment. After 24 hours it decreased significantly, but increased again at the final period. Renal vascular resistance increased almost 3 folds at 2 hours after envenomation, and returned to the control range around 24 to 48 hours.

Changes in renal function

According to the increase of renal vascular resistance due to the effect of the venom, renal fraction decreased significantly from 17 $\pm$ 4 % to 11 $\pm$ 5 % ($P < 0.05$) at 2 hours after envenomation (table 4), and contimue decreased significantly until the end of the experiment ($P < 0.01$). The effective renal blood flow decreased significantly from 267 ± 73 ml./min. to 173 ± 94 ml./min. ($P < 0.05$) at the first 2 hours, then increased to 182 ± 33 ml./min. and 212 ± 88 ml./min. at 24 and 48 hours after envenomation respectively. The similar changes in effective renal plasma flow were observed after venom injection (figure 5).

The glomerular filtration rate (GFR) decreased from 43 ± 7 ml./min. to 37 ± 16 ml./min. at 2 hours after venom injection, and further decreased significantly to 30 ± 12 ml./min. ($P < 0.05$) at 24 hours. During the second day of venom injection GFR increased slightly to 40 ± 13 ml./min. as compared with the control level. The decrease of effective renal plasma flow was more than the decrease of GFR, therefore, the filtration fraction increased significantly ($P < 0.01$) after envenomation (figure 5).

Owing to the determination of the tubular activities (table 4 and figure 6), it was found that transport of PAH decreased insignificantly at 2 to 24 hours of the experimental period, and returned to the control level again at the end of the experiment. There was no significant difference in plasma creatinine concentrations between the control and the experimental periods, as shown in figure 8. Plasma osmolality increased from 295 ± 9 to 299 ± 7 mOsm./l. at 2 hours after venom injection. At 24 hours after envenomation it increased to 310 ± 11 mOsm./l. and decreased again to 301 ± 14 mOsm./l. at the second day of the experiment. Osmolar clearance

Table 4 Effect of Russell's viper venom on renal parameters in dogs (Mean ± S.D. for 8 dogs)

	control	2 hours	24 hours	48 hours
R.V.R. (10^3 dyne-sec./cm.^{5})	43.363 ± 14.968	113.603 ±127.515	59.516 ± 31.966	68.447 ± 67.606
R.F. (%)	17.20 ± 4.15	11.21* ± 5.29	11.44 ± 7.87	12.70** ± 6.22
R.P.F. (ml./min.)	179.20 ± 53.63	106.03* ± 59.78	130.41 ± 59.48	157.05 ± 65.64
R.B.F. (ml./min.)	267.28 ± 73.05	173.74* ± 94.76	182.49* ± 83.07	212.36 ± 88.79
G.F.R. (ml./min.)	43.16 ± 7.28	37.20 ± 16.57	30.63* ± 12.49	40.34 ± 13.66
U.F. (ml./min.)	1.06 ± 0.56	0.47** ± 0.30	0.51* ± 0.20	0.65 ± 0.41
F.F. (%)	24.91 ± 3.72	37.62** ± 7.37	25.01 ± 7.39	27.99 ± 8.43
T. PAH (mg./min.)	6.217 ± 1.893	4.831 ± 3.026	4.706 ± 2.668	6.163 ± 3.336
P. creatinine (ugm./ml.)	3.321 ± 1.583	3.054 ± 2.472	7.554 ± 7.577	5.946 ± 3.378
P_{Osm} (mOsm./lit.)	295.50 ± 9.37	299.87 ± 7.32	310.87* ± 11.59	301.12 ± 14.18
U_{Osm} (mOsm./lit.)	419.75 ± 121.51	429.62 ± 106.12	925.87** ± 298.08	997.12** ± 356.83
C_{Osm} (ml./min.)	1.35 ± 0.38	0.98*** ± 0.49	1.13 ± 0.56	1.30 ± 0.70
C_{H_2O} (ml./min.)	- 0.29 ± 0.32	- 0.12 ± 0.25	- 0.92* ± 0.46	- 1.17** ± 0.49

Values were statistically significantly different from the control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

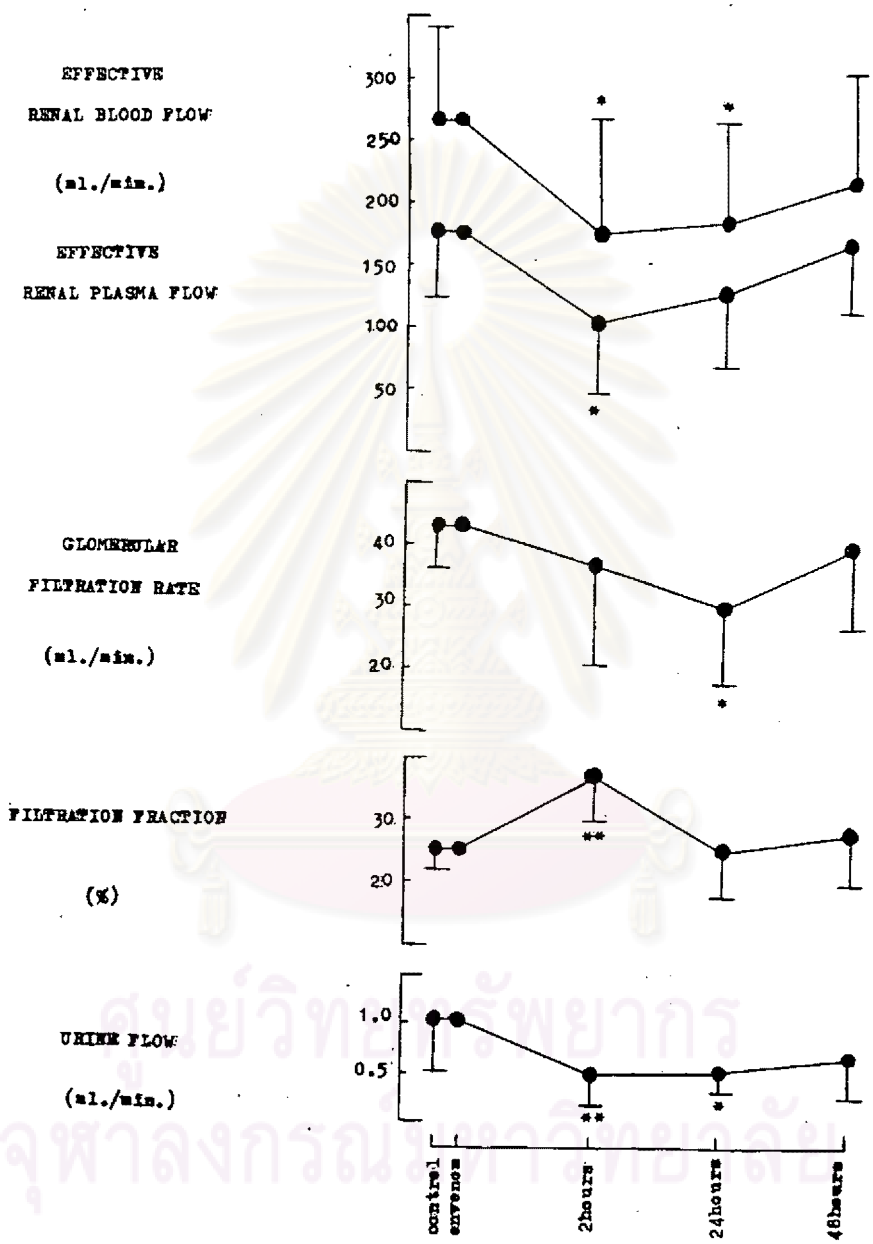


Figure 5 Effect of Russell's viper venom on effective renal blood flow, effective renal plasma flow, glomerular filtration rate, filtration fraction and urine flow. (Mean and S.D. of 6 dogs)
* P(0.05 , ** P(0.01 , *** P(0.001

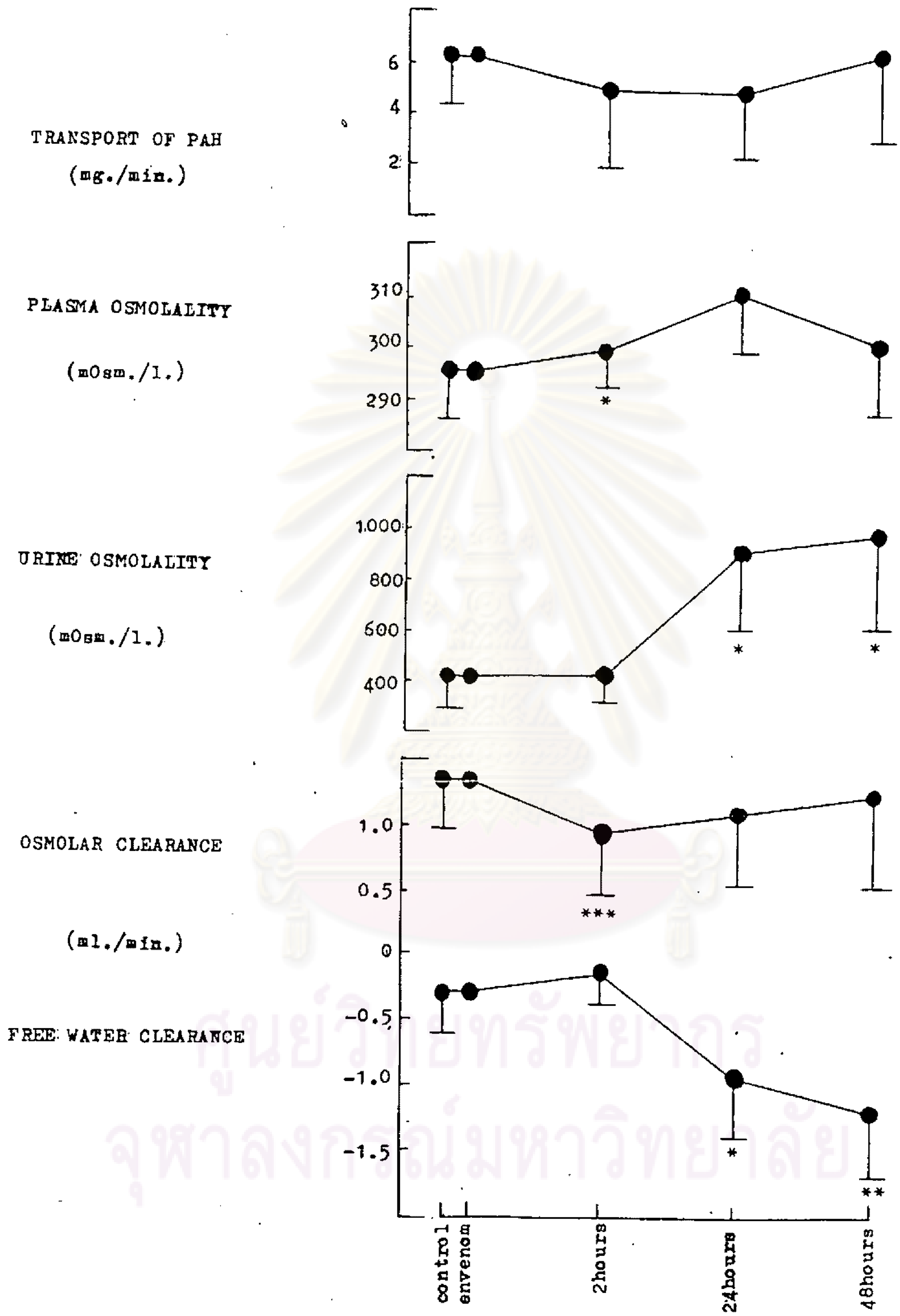


Figure 6 Effect of Russell's viper venom on transport of PAH, plasma osmolality, urine osmolality, osmolar clearance and free water clearance. (Mean and S.D. of 8 dogs)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

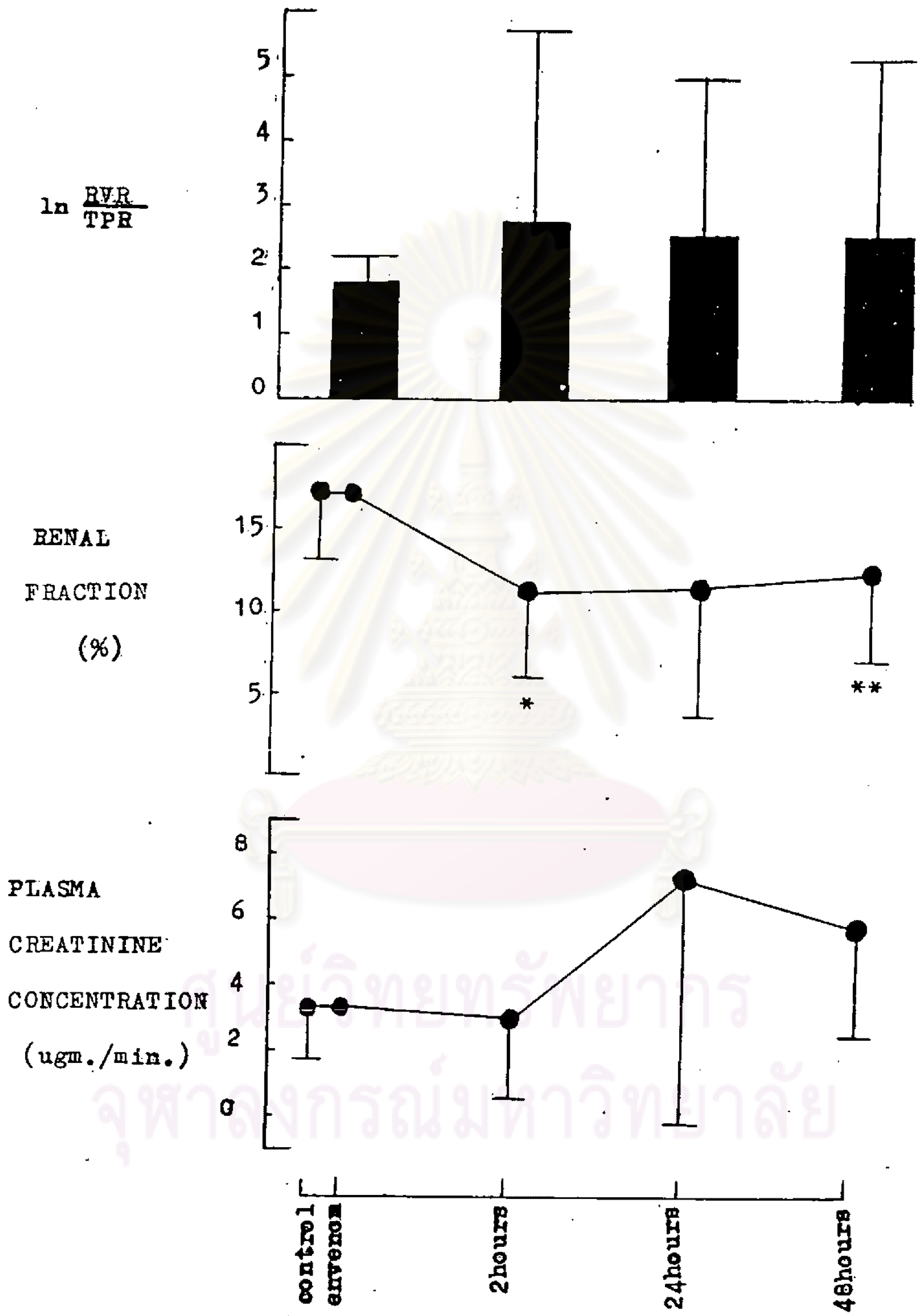


Figure 7 Effect of Russell's viper venom on ln $\frac{RVR}{TPR}$, renal fraction and plasma creatinine. (Mean and S.D. of 8 dogs)

* $P < 0.05$, ** $P < 0.01$

decreased significantly at the first period from 1.3 $\pm$ 0.3 to 0.9 $\pm$ 0.4 ml./min. ($P < 0.001$), and then gradually increased to 1.3 $\pm$ 0.6 ml./min. at the end of the experiment. The free water clearance increased slightly at the first period whereas it decreased significantly ($P < 0.01$) at 24 hours till the end of the experiment.

Effects of Russell's viper venom on plasma and urinary electrolyte concentrations are shown in table 5. Filtered load of these electrolytes decreased throughout the experiment, as compared with the control. However they decreased significantly ($P < 0.05$ and $P < 0.01$) (table 5) at 24 hours after venom injection. At the end of the experiment, they had a tendency to increase to the control level. It was found that plasma sodium concentration increased significantly from 139 $\pm$ 3 to 141 $\pm$ 2 mEq./l. ($P < 0.05$) at 24 hours after envenomation. The urinary excretion of sodium decreased significantly from 125 $\pm$ 39 to 39 $\pm$ 26 uEq./min. ($P < 0.001$) at 2 hours after venom injection, then gradually increased to the control level at the end of the experiment. The fractional excretion of sodium altered in the similar pattern as its excretion. Plasma potassium concentration was constant throughout the experiment, while urinary potassium concentration increased after envenomation. Urinary excretion and fractional excretion of potassium decreased at 2 hours after envenomation, but were not significantly different when compared with the control period. The plasma chloride concentration changed insignificantly after envenomation, but its excretion and

Table 5 Effect of Russell's viper venom on urinary electrolytes excretion of dogs. (Mean ± S.D. for 8 dogs)

		plasma concentration (mEq./lit.)	urine concentration (mEq./lit.)	filtered load (mEq./min.)	excretion (uEq./min.)	excretion fraction (%)
Na^+	control	139.37 ± 3.96	132.37 ± 45.70	6.00 ± 0.91	125.25 ± 39.71	2.16 ± 0.82
	2 hours	139.62 ± 3.42	88.62 ± 42.65*	5.19 ± 2.35	39.83 ± 26.34***	0.89 ± 0.58**
	24 hours	141.75 ± 2.19*	147.87 ± 70.31	4.33 ± 1.75*	78.13 ± 53.25	1.97 ± 1.24
	48 hours	137.50 ± 4.31	204.87 ± 87.90	5.51 ± 1.77	119.57 ± 82.59	2.42 ± 1.77
K^+	control	3.70 ± 0.41	26.25 ± 12.15	0.16 ± 0.03	25.03 ± 10.70	15.69 ± 5.99
	2 hours	3.30 ± 0.34	43.37 ± 25.02*	0.12 ± 0.06	16.93 ± 8.78	14.83 ± 4.48
	24 hours	3.70 ± 0.49	60.25 ± 40.62	0.11 ± 0.05**	27.17 ± 12.62	27.54 ± 15.65
	48 hours	3.60 ± 0.40	44.62 ± 19.51	0.14 ± 0.04	24.42 ± 12.51	18.16 ± 9.94
Cl^-	control	119.62 ± 2.20	174.75 ± 54.47	5.17 ± 0.89	164.53 ± 46.66	3.31 ± 1.16
	2 hours	121.75 ± 3.01	68.36 ± 48.20**	4.50 ± 1.98	30.27 ± 23.57***	0.82 ± 0.70***
	24 hours	123.87 ± 4.64	158.50 ± 112.88	3.81 ± 1.61*	87.13 ± 85.56*	2.28 ± 2.05
	48 hours	119.62 ± 13.15	232.12 ± 117.40	4.72 ± 1.41	146.23 ± 132.91	3.33 ± 2.79
Ca^{++}	control	5.09 ± 0.71	2.34 ± 0.95	0.22 ± 0.04	2.21 ± 0.97	1.06 ± 0.54
	2 hours	4.83 ± 0.61	2.22 ± 2.06	0.18 ± 0.08	0.82 ± 0.27**	0.59 ± 0.41
	24 hours	4.93 ± 0.37	2.82 ± 2.20	0.15 ± 0.06*	1.31 ± 0.67*	1.02 ± 0.68
	48 hours	4.72 ± 0.46	3.11 ± 2.19	0.19 ± 0.07	1.91 ± 1.21	1.13 ± 0.80
Pi	control	3.83 ± 0.57	18.32 ± 15.45	1.66 ± 0.42	0.16 ± 0.10	9.44 ± 5.15
	2 hours	3.51 ± 1.15	18.91 ± 3.37	1.29 ± 0.64	0.06 ± 0.08	4.34 ± 5.20
	24 hours	3.85 ± 1.02	51.15 ± 27.81*	1.09 ± 0.28**	0.25 ± 0.15	23.74 ± 13.18*
	48 hours	3.74 ± 0.84	33.90 ± 35.96	1.49 ± 0.51	0.17 ± 0.15	18.42 ± 12.68

Values were statistically significantly different from the control. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Remark: concentration of Pi used mg.%, filter load and excretion of Pi used mg./min.

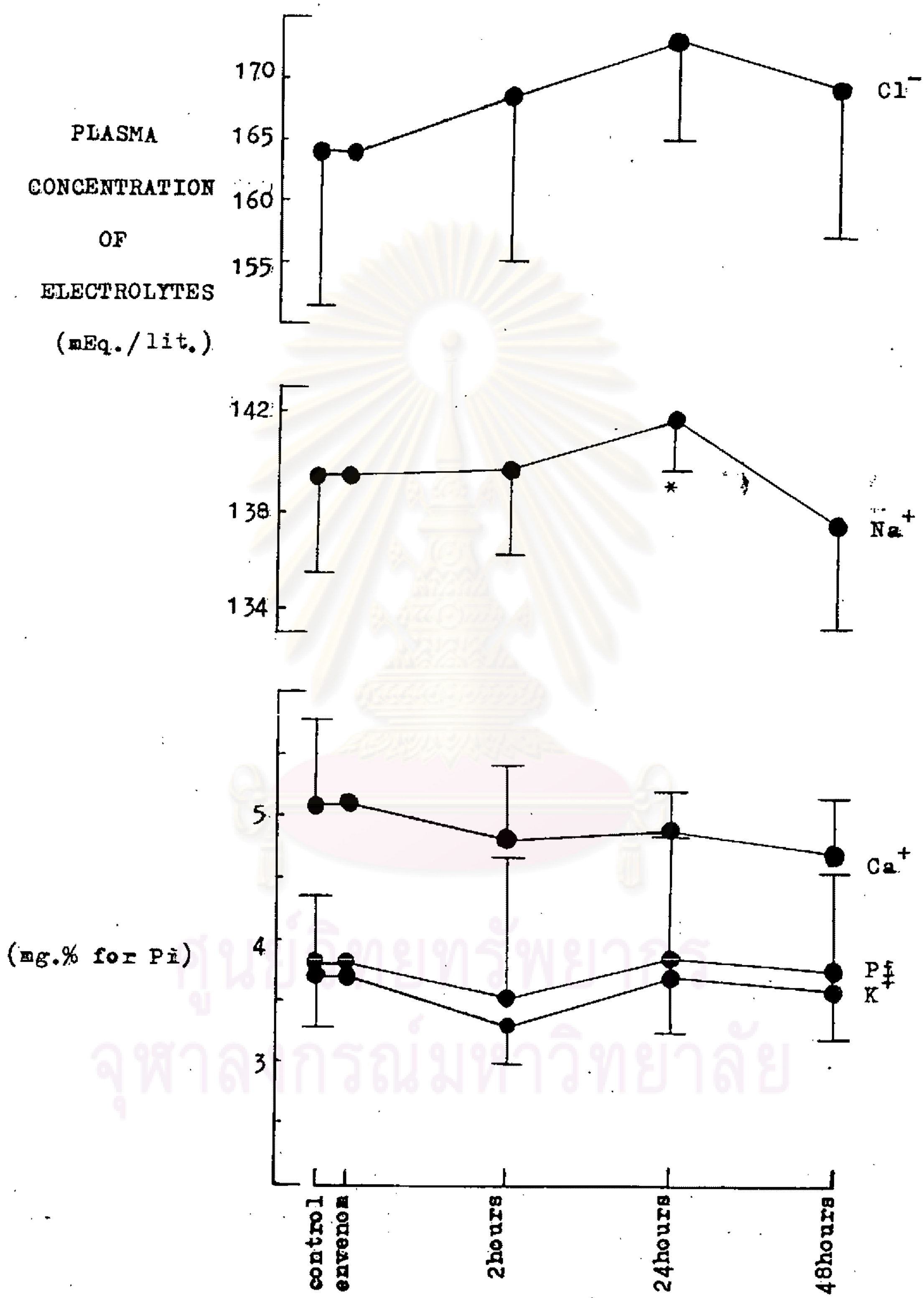


Figure 8 Effect of Russell's viper venom on plasma concentration of electrolytes. (Mean and S.D. of 8 dogs)

* $P<0.05$

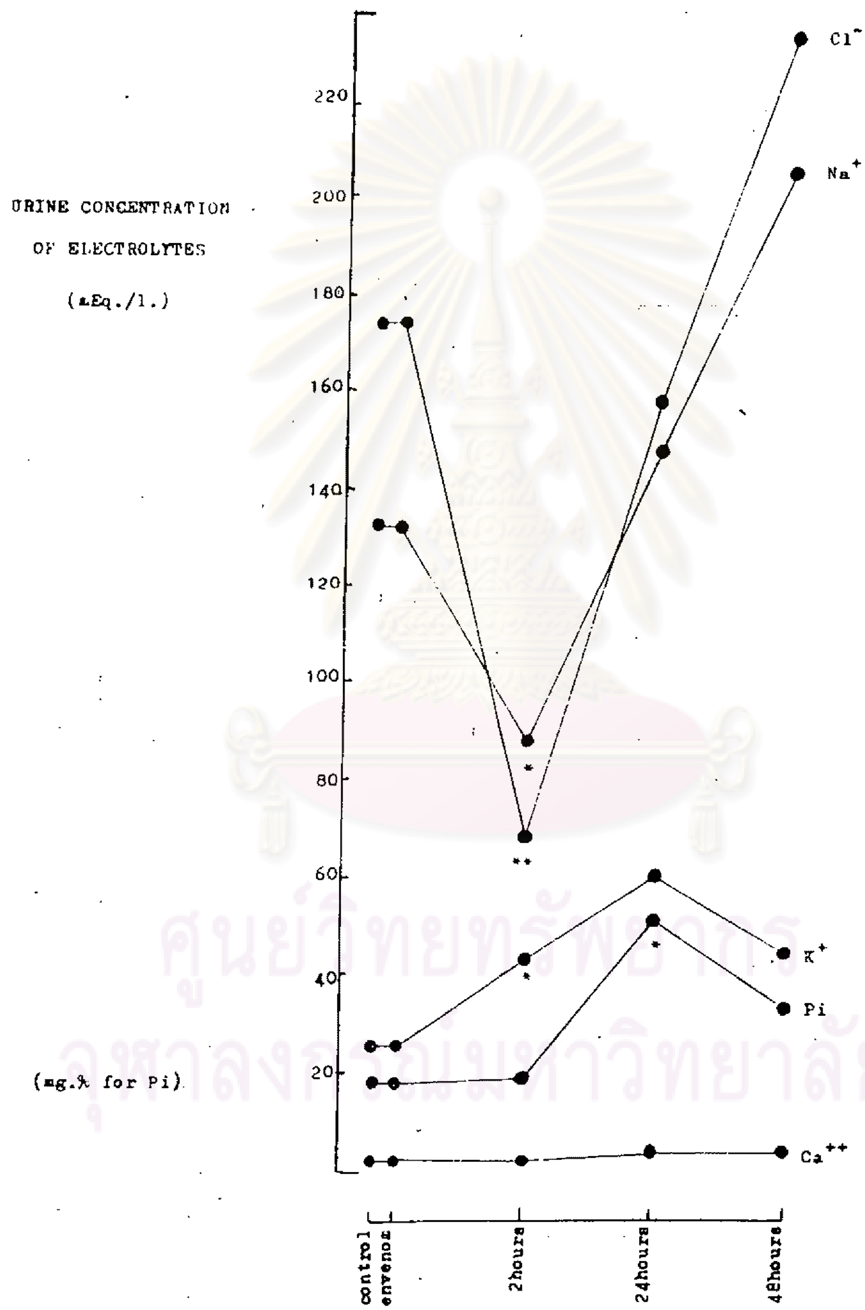


Figure 9 Effect of Russell's viper venom on urine concentration of electrolytes. (Mean of 8 dogs) * $P<0.05$, ** $P<0.01$

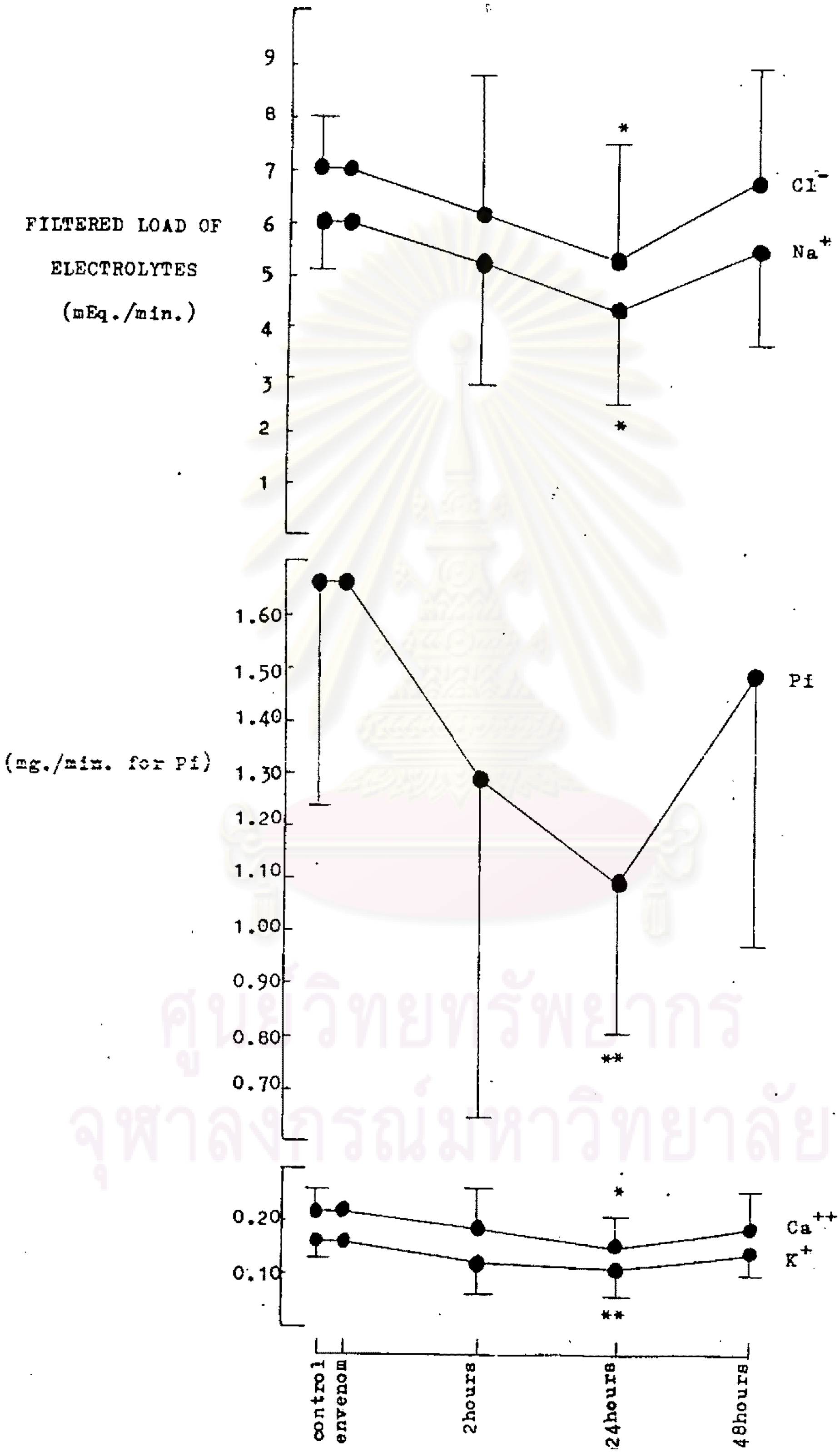


Figure 10 Effect of Russell's viper venom on filtered load of electrolytes. (Mean and S.D. of 8 dogs) * $P < 0.05$, ** $P < 0.01$

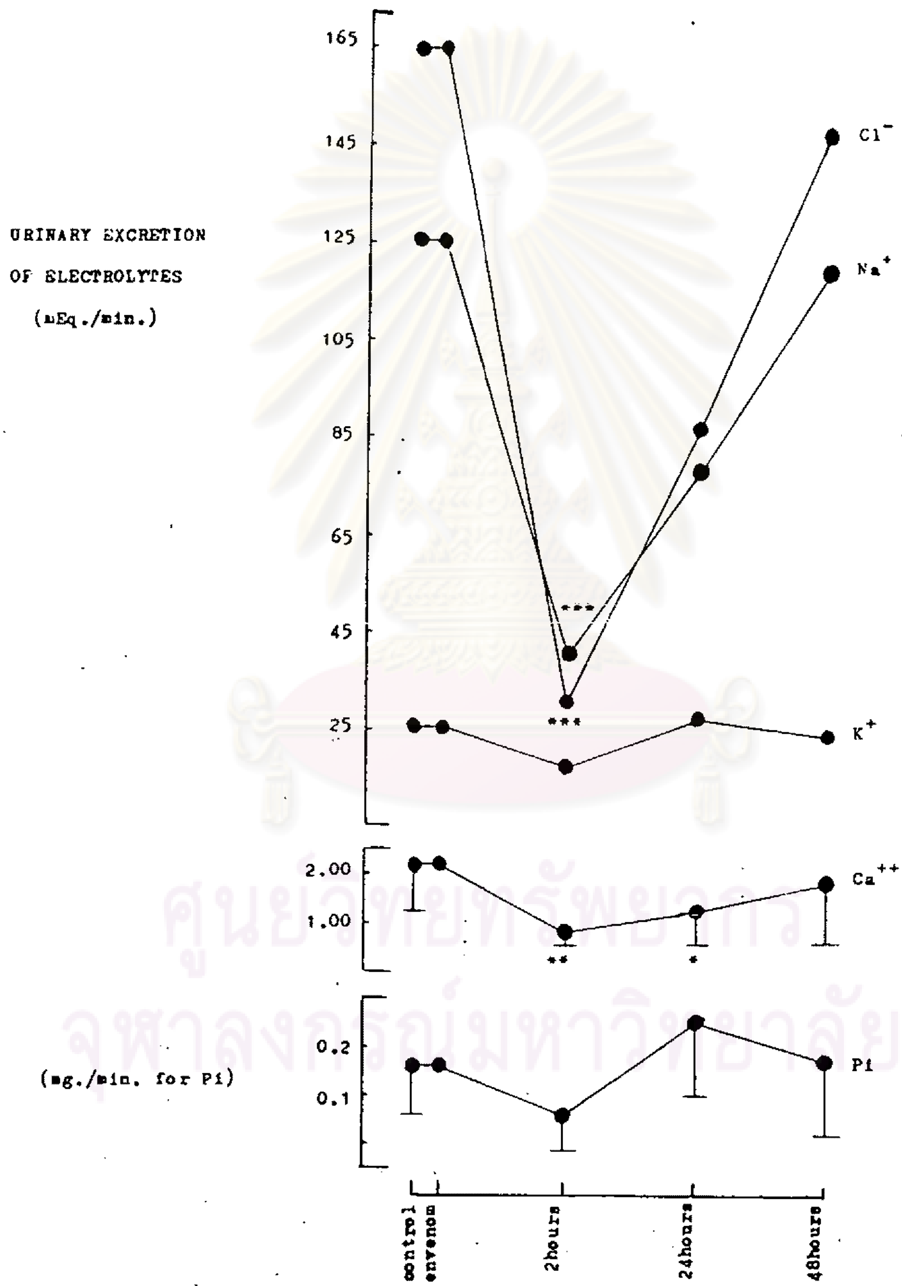


Figure 11 Effect of Russell's viper venom on urinary excretion of electrolytes. (Mean of 8 dogs) * $P<0.05$, ** $P<0.01$, *** $P<0.001$

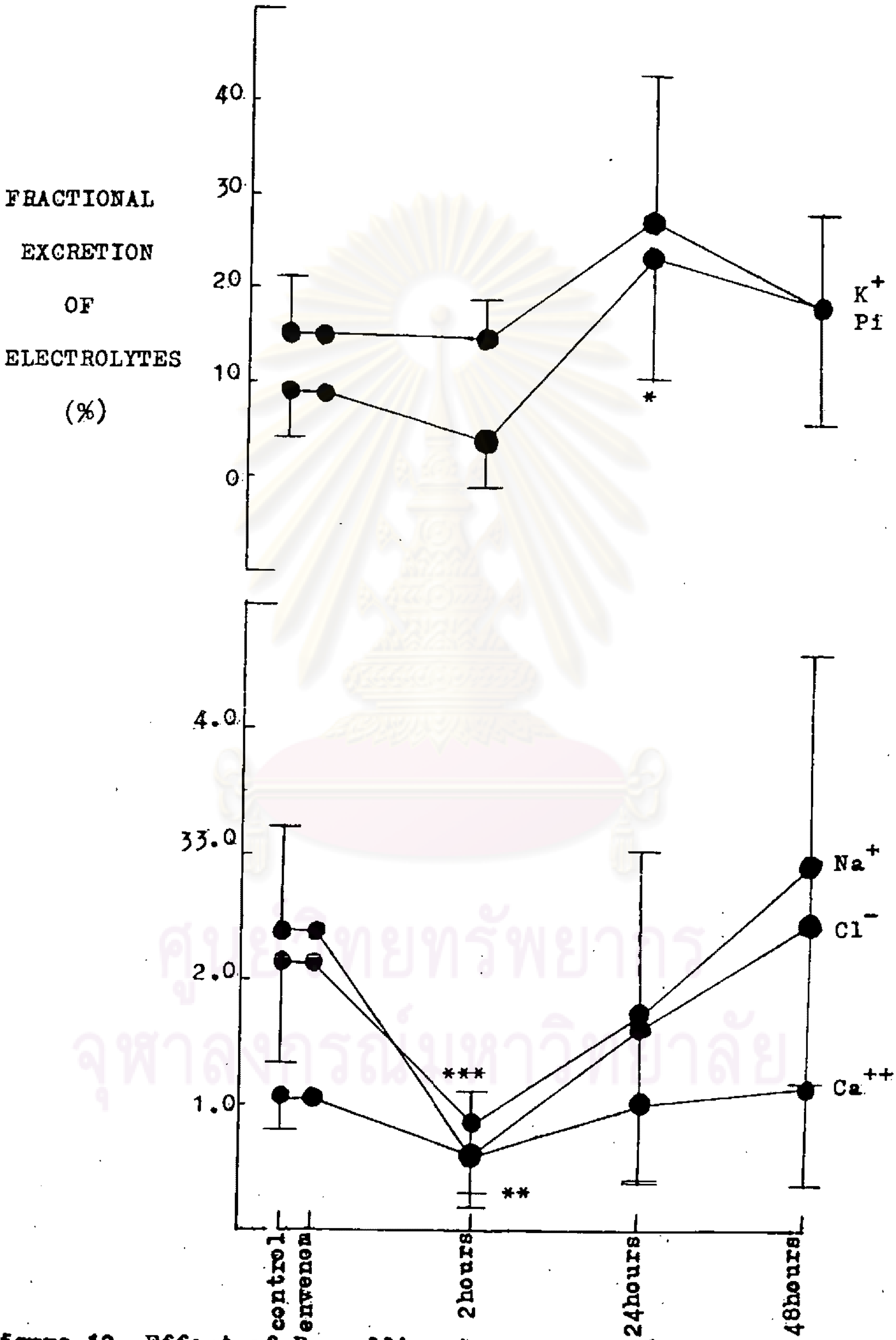


Figure 12 Effect of Russell's viper venom on fractional excretion of electrolytes. (Mean and S.D. of 8 dogs)
* P(0.05 , ** P(0.01 , *** P(0.001

fractional excretion decreased significantly ($P < 0.001$) at 2 hours after envenomation, from 164 $\pm$ 46 to 30 $\pm$ 23 uEq./min. and from 3.3 $\pm$ 1.1 to 0.8 $\pm$ 0.7 % respectively. After these decrement, the urinary excretion and fractional excretion of chloride increased gradually at 24 and 48 hours of the experiment. Plasma calcium concentration increased slightly throughout the experiment. The urinary excretion of calcium decreased significantly from 2.2 $\pm$ 0.9 to 0.8 $\pm$ 0.2 mEq./min. ($P < 0.01$) at 2 hours after venom injection. Changes of fractional excretion of calcium was also observed after envenomation. After venom injection, plasma phosphorus concentration was constant, even though urinary phosphorus cocentration was significantly increased at 24 hours, while its excretion also increased nearly 75 % of control. Therefore fractional excretion of phosphorus at 24 hours increased significantly from 4.3 $\pm$ 5 to 23.7 $\pm$ 13 %