



Chapter IV

Results

Changes in plasma thyroxine concentration and the weight of kidneys in normal, hyperthyroid and hypothyroid rats given Russell's viper venom.

Plasma Thyroxine Concentration :

The present data show that the concentration of plasma thyroxine in rats given exogenous L-thyroxine (T₄ group) increased significantly from $4.38 \pm 0.54 \mu\text{g/dl}$ in normal rats (N group) to $5.95 \pm 0.81 \mu\text{g/dl}$ ($p < 0.05$). In thyroidectomized rats (TX group) plasma thyroxine concentration decreased significantly from $4.38 \pm 0.54 \mu\text{g/dl}$ in normal rats to $1.16 \pm 0.85 \mu\text{g/dl}$ ($p < 0.001$), which decreased by approximately 74% (Table 1 and Fig.2).

The results summarized in Table 1 and Fig.3 show that there was no significant change in plasma thyroxine concentration in all groups of rat received the venom injection when compared to their normal conditions received normal saline injection.

The Weight of Kidneys :

The weight of kidneys of hyperthyroid rats significantly increased when compared to normal rats ($p < 0.01$) (Table 1 and Fig.2). No alterations were recorded in hypothyroid rats. There were no changes

Table 1 Effect of subcutaneous injection of Russell's viper venom on plasma thyroxine concentration and the weight of kidneys in normal (N) hyperthyroid (T_4) and hypothyroid (TX) rats.

(Mean \pm S.D.)

	N	N + Venom	T_4	T_4 + Venom	TX	TX + Venom
$P_{\text{thyroxine}}$ ($\mu\text{g/dl}$)	4.38 ± 0.54	4.45 ± 0.50	5.95* ± 0.81	5.02 ± 0.82	1.16*** ± 0.85	1.16 ± 0.88
Kidneys Wt. (g/100g BW)	0.65 ± 0.05	0.68 ± 0.09	0.71** ± 0.03	0.69 ± 0.13	0.59 ± 0.08	0.66 ± 0.09

P-value with respect to normal rats, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

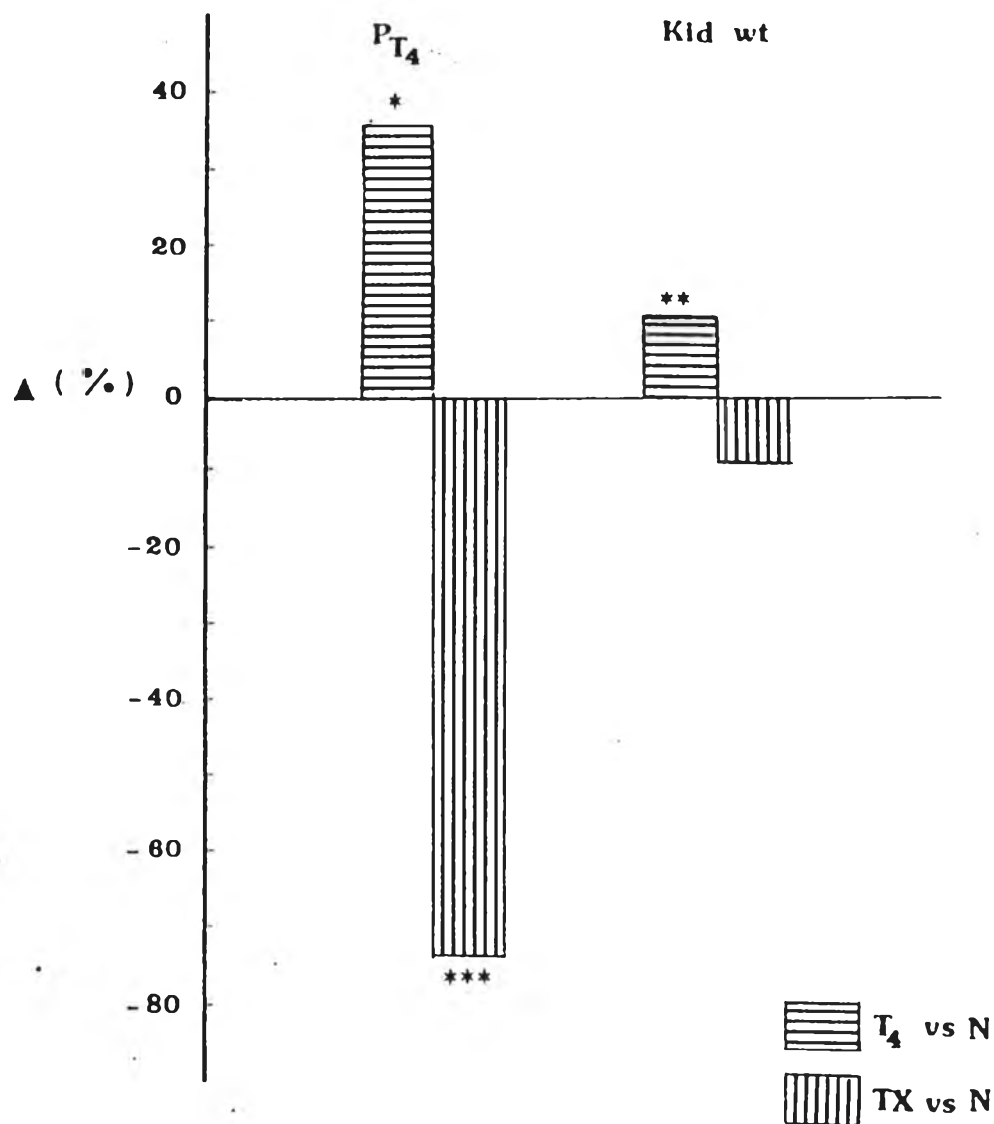


Fig.2 Percent changes of plasma thyroxine concentration and the weight of kidneys in hyperthyroid (T₄) and hypothyroid (TX) rats in comparison to normal (N) rats. P-value with respect to normal rats, *P < 0.05, **P < 0.01, *** P < 0.001.

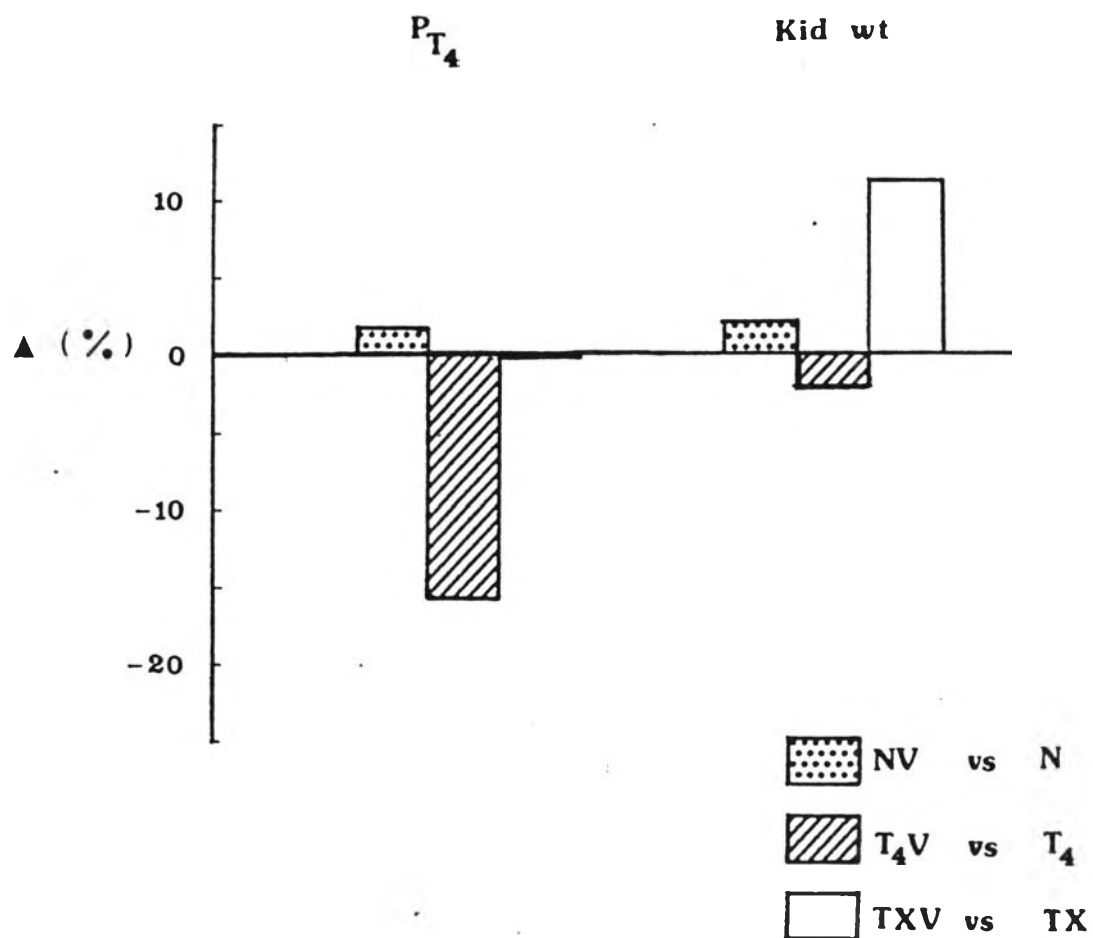


Fig. 3 Percent changes of plasma thyroxine concentration and the weight of kidneys in normal, hyperthyroid and hypothyroid rats with and without the Russell's viper venom administration.

P-value with respect to the condition without the Russell's viper venom administration.

appeared in normal and thyroidectomized rats received the venom injection when compared to the condition without given the venom (Table 1 and Fig.3).

Changes in general circulation in normal, hyperthyroid and hypothyroid rats given Russell's viper venom.

General Circulation :

It was found that heart rate (HR), mean arterial blood pressure (MABP) and pulse pressure (PP) in hyperthyroid rats increased significantly by approximately 22%, 20% and 31% respectively when compared with normal rats while hematocrit (Hct) did not change significantly (Table 2 and Fig.4). In thyroidectomized rats, heart rate, mean arterial blood pressure and pulse pressure decreased significantly by approximately 22%, 14% and 26% respectively. Hematocrit as the same in hyperthyroid rats, was not different from normal rats.

Animals in all groups given Russell's viper venom caused a reduction of hematocrit when compared with the condition without the venom, while pulse pressure did not change significantly in these groups (Table 2 and Fig.5). In hyperthyroid rats given the venom (T_4V group), heart rate decreased by approximately 19% from 509 ± 8 beats/min in T_4 group to 415 ± 36 beats/min ($p < 0.001$). While in normal and thyroidectomized rats given the venom, heart rate did not change when compared to N group and TX group respectively. Mean arterial blood pressure decreased significantly in T_4V

Table 2 Effect of subcutaneous injection of Russell's viper venom on general circulation in normal (N), hyperthyroid (T₄) and hypothyroid (TX) rats. (Mean \pm S.D.)

	N	N + Venom	T ₄	T ₄ + Venom	TX	TX + Venom
HR (beat/min)	416 \pm 21	436 \pm 16	509 ^{***} \pm 8	415 ^{***} \pm 36	326 ^{***} \pm 15	316 \pm 15
MABP (mmHg)	136.25 \pm 13.24	122.31 \pm 17.14	163.96 ^{***} \pm 4.62	133.13 ^{***} \pm 9.70	117.29 [*] \pm 12.50	106.11 \pm 19.40
PP (mmHg)	31.88 \pm 5.94	36.88 \pm 17.51	41.88 [*] \pm 10.33	35.63 \pm 3.20	23.75 ^{**} \pm 3.54	23.33 \pm 6.83
Hct (%)	45.44 \pm 3.06	32.44 ^{**} \pm 8.70	49.81 \pm 1.13	38.56 ^{***} \pm 5.19	46.75 \pm 2.83	31.75 ^{**} \pm 9.94

P-value with respect to normal rats, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

P-value with respect to the condition without the Russell's viper venom administration, ** $P < 0.01$, *** $P < 0.001$.

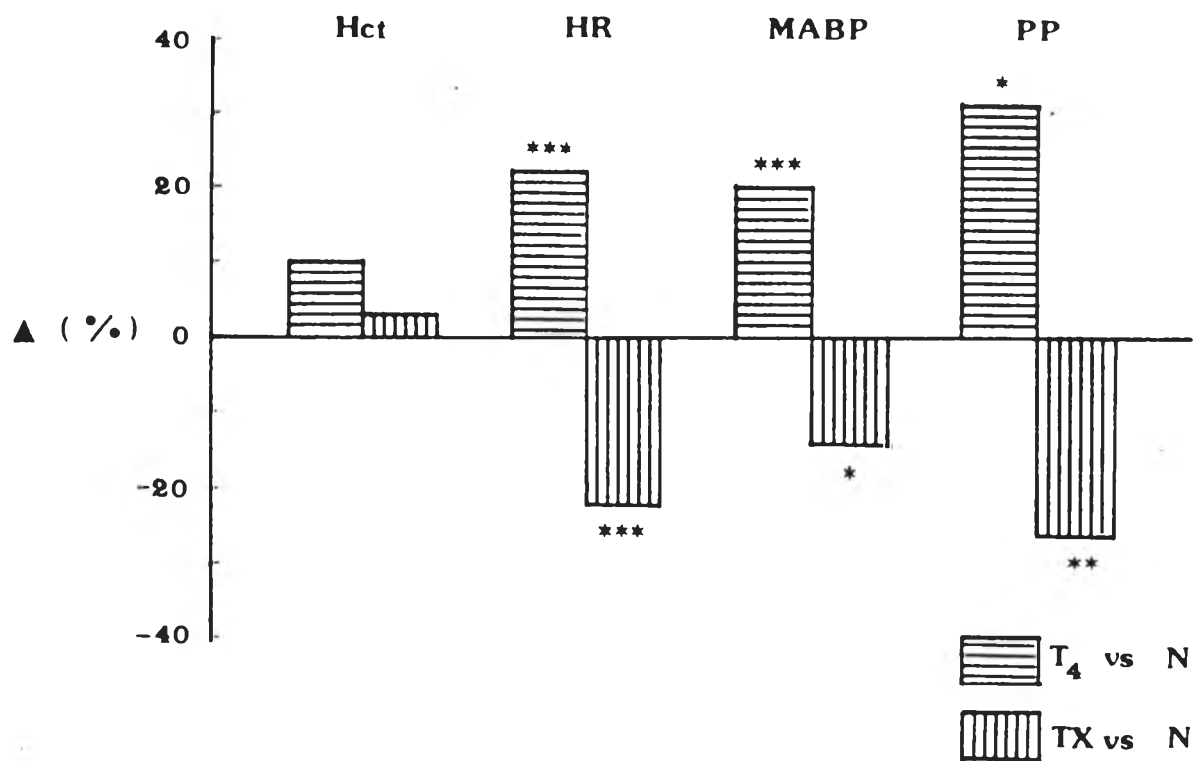


Fig. 4 Percent changes of general circulation in hyperthyroid and hypothyroid rats in comparison to normal rats .
P-value with respect to normal rats , * $P < 0.05$, ** $P < 0.01$,
*** $P < 0.001$.

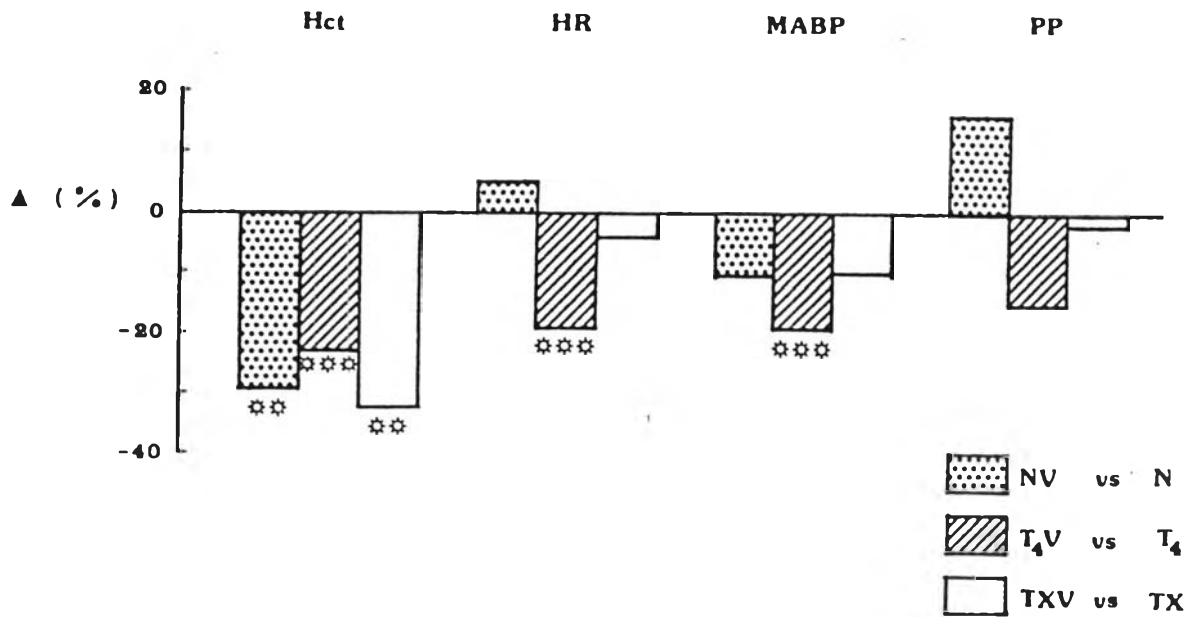


Fig. 5

Percent changes of general circulation in normal, hyperthyroid and hypothyroid rats with and without the Russell's viper venom administration.

P-value with respect to the condition without the Russell's viper venom administration, ** $P < 0.01$, *** $P < 0.001$.

group from 163.96 ± 4.62 mmHg in T₄ group to 133.13 ± 9.70 mmHg ($p < 0.001$), by approximately 19%. There were no significant alterations of mean arterial blood pressure in NV and TXV group.

Changes in renal hemodynamic and renal function in normal, hyperthyroid and hypothyroid rats given Russell's viper venom.

Renal Hemodynamic :

The results summarized in Table 3 and Fig.6 show marked reduction in glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and effective renal blood flow (ERBF) in hypothyroid rats, nearly 59% in all three parameters ($p < 0.001$) when compared to the normal rats. A slight reduction was observed in hyperthyroid rats. Renal vascular resistance (RVR) of thyroidectomized rats increased significantly ($p < 0.001$) by approximately 191% when compared to intact rats. No significant alteration in renal vascular resistance appeared in hyperthyroid rats. Filtration fraction (FF) did not change in both hyperthyroid and hypothyroid rats when compared with the normal rats.

The data in Table 3 and Fig.7 reveal the effect of Russell's viper venom on the renal hemodynamics. The significant reduction of glomerular filtration rate, effective renal plasma flow, effective renal blood flow and filtration fraction were apparent in the normal rats that received the venom injection when compared to the condition without given the venom, while the marked increment in renal vascular resistance

Table 3 Effect of subcutaneous injection of Russell's viper venom on renal hemodynamic in normal (N), hyperthyroid (T₄) and hypothyroid (TX) rats. (Mean \pm S.D.)

	N	N + Venom	T ₄	T ₄ + Venom	TX	TX + Venom
GFR (ml/min/g kid. wt)	1.22 \pm 0.26	0.50 ^{***} \pm 0.20	1.05 \pm 0.16	1.19 \pm 0.18	0.51 ^{***} \pm 0.26	0.07 ^{***} \pm 0.03
ERPF (ml/min/g kid wt)	4.95 \pm 1.43	3.14 [*] \pm 1.01	4.24 \pm 0.70	4.28 \pm 1.09	2.00 ^{***} \pm 0.86	0.21 ^{***} \pm 0.11
ERBF (ml/min/g kid wt)	9.00 \pm 2.25	4.81 ^{**} \pm 1.90	8.43 \pm 1.26	7.09 \pm 2.07	3.72 ^{***} \pm 1.51	0.30 ^{***} \pm 0.12
FF (%)	+ 25.41 \pm 4.11	16.15 ^{***} \pm 3.88	25.37 \pm 5.12	31.61 \pm 8.38	25.58 \pm 7.86	40.41 \pm 19.07
RVR (10 ³ dyne sec/cm ⁵)	616.07 \pm 196.59	1112.46 [*] \pm 528.08	801.10 \pm 163.87	862.63 \pm 218.19	1793.86 ^{***} \pm 656.64	19080.78 ^{***} \pm 12397.98

P-value with respect to normal rats, *** P < 0.001.

P-value with respect to the condition without the Russell's viper venom administration, * P < 0.05, ** P < 0.01, *** P < 0.001.

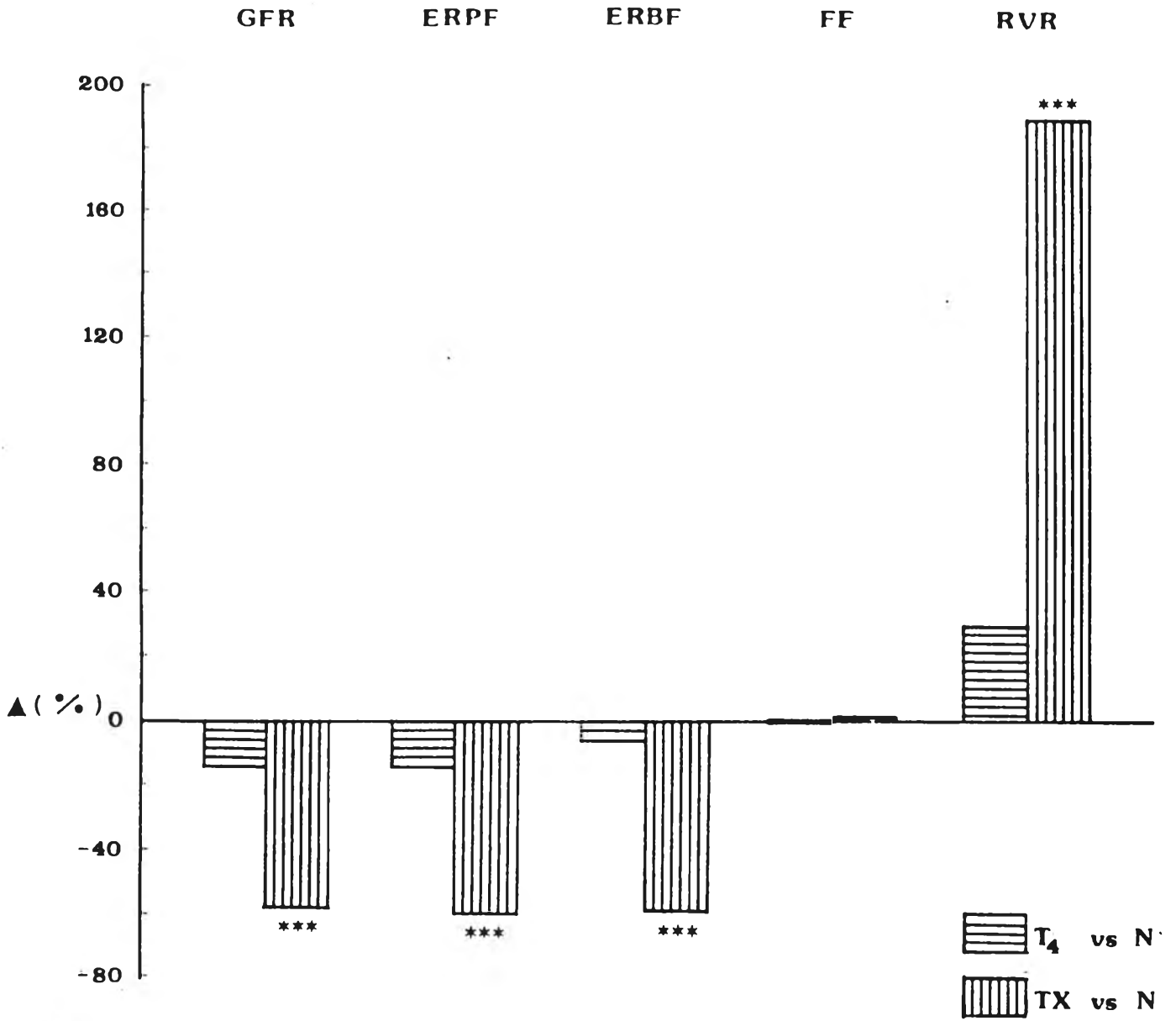


Fig. 6 Percent changes of renal hemodynamic in hyperthyroid and hypothyroid rats in comparison to normal rats. P-value with respect to normal rats, *** $P < 0.001$.

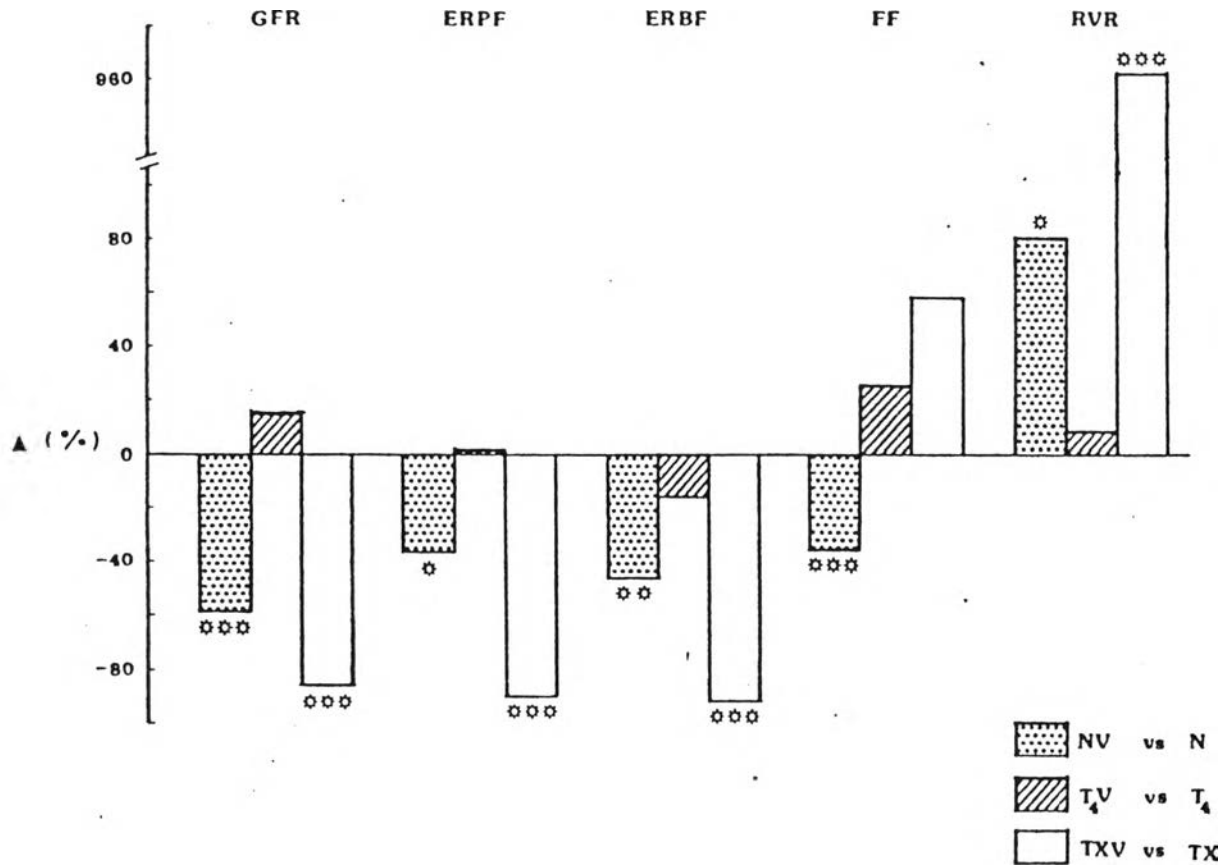


Fig. 7 Percent changes of renal hemodynamic in normal, hyperthyroid and hypothyroid rats with and without the Russell's viper venom administration. P-value with respect to the condition without the Russell's viper venom administration, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

was observed. In hyperthyroid rats that received the venom injection, all the renal hemodynamic parameters did not change significantly when compared to hyperthyroid rats given normal saline. There were significant decreased in glomerular filtration rate, effective renal plasma flow and effective renal blood flow ($p < 0.001$) in thyroidectomized rats that received the venom injection, lower by approximately 86%, 90% and 92% respectively compared to thyroidectomized rats given normal saline, whereas a sharp increment in renal vascular resistance was observed ($p < 0.01$), nearly ten folds higher than the condition without given the venom. There was not statistically increment in filtration fraction in this group.

Renal Function :

Data summarized in Table 4 and Fig.8 show that the rate of urine flow (V) was not affected neither by L-thyroxine injection nor thyroidectomy and the responses of osmolar clearance (C_{osm}) and free water clearance (C_{H_2O}) to L-thyroxine injection were also similar to the rate of urine flow. In thyroidectomized rats, osmolar clearance decreased significantly ($p < 0.001$) by 65% lower than that of the normal rats while free water clearance increased significantly ($p < 0.05$).

The rate of urine flow was affected by the venom only in hyperthyroid rats. It increased significantly from $33.69 \pm 13.62 \mu\text{l}/\text{min}$ in T_4 group to $89.52 \pm 39.41 \mu\text{l}/\text{min}$ ($p < 0.005$) in T_4V group by approximately 166% while the rate of urine flow in NV and TXV group did not change significantly when compared to N and TX group respectively (Table 4 and

Fig.9). There was no significant decreased in osmolar clearance observed in NV and T_4V group, whereas significant decrement ($p < 0.05$) recorded in TXV group, lower by approximately 60% from TX group. Free water clearance showed significant increased ($p < 0.05$) in T_4V group and did not change significantly in NV and TXV group when compared to the condition given normal saline solution.

Urinary excretion of all electrolytes decreased significantly ($p < 0.01$) in thyroidectomized rats. In hyperthyroid rats, the electrolytes showed no significant alteration in urinary excretion (Table 4 and Fig.10).

It was found that urinary excretion of the electrolytes observed in this study in T_4V group were not affected by the venom (Fig.11). In NV group, urinary excretion of potassium, chloride and calcium decreased significantly, lower by approximately 40%, 22% and 42% respectively when compared to N group while urinary excretion of sodium did not change significantly. Urinary excretion of potassium and chloride in TXV group were similarly depressed which differed by approximately 67% and 60% respectively from TX group while urinary excretion of sodium and calcium did not change significantly.

Plasma osmolality (P_{osm}) slightly decreased in hyperthyroid rats, but significantly decreased ($p < 0.05$) in hypothyroid rats. There were no alteration in plasma electrolytes concentration in hyperthyroid rats. In hypothyroid rats, plasma sodium and chloride decreased significantly while plasma potassium increased significantly and did not have any changes in

Table 4 Effect of subcutaneous injection of Russell's viper venom on the rate of urine flow, urinary excretion of electrolytes, osmolar clearance and free water clearance in normal (N), hyperthyroid (T_4) and hypothyroid (TX) rats. (Mean \pm S.D.)

	N	N + Venom	T_4	T_4 + Venom	TX	TX + Venom
V	51.67	49.84	33.69	89.52 ^{☆☆}	39.31	6.55
(μ l/min)	\pm 38.19	\pm 40.11	\pm 13.62	\pm 39.41	\pm 47.02	\pm 4.72
$U_{Na} V$	4.29	3.77	4.87	4.35	0.20 ^{***}	0.20
(μ Eq/min)	\pm 1.84	\pm 1.03	\pm 1.36	\pm 2.04	\pm 0.12	\pm 0.18
$U_k V$	2.78	1.67 [*]	1.86	2.42	0.76 ^{***}	0.25 ^{☆☆☆}
(μ Eq/min)	\pm 1.04	\pm 0.46	\pm 0.93	\pm 0.85	\pm 0.29	\pm 0.08
$U_{Cl} V$	5.93	4.62 [*]	6.04	5.88	0.57 ^{***}	0.23 [*]
(μ Eq/min)	\pm 1.18	\pm 0.73	\pm 1.67	\pm 3.22	\pm 0.40	\pm 0.04
$U_{Ca} V$	1.02	0.59 ^{☆☆}	1.27	1.40	0.54 [*]	0.25
(μ g/min)	\pm 0.34	\pm 0.12	\pm 0.36	\pm 0.80	\pm 0.40	\pm 0.09
C_{Osm}	83.14	57.62	87.29	81.96	29.50 ^{***}	11.73 [*]
(μ l/min)	\pm 25.25	\pm 31.75	\pm 21.34	\pm 29.65	\pm 17.38	\pm 6.02
C_{H_2O}	- 31.46	- 7.78	- 53.61	+ 7.59 [*]	+ 9.81 [*]	- 5.20
(μ l/min)	\pm 38.22	\pm 16.62	\pm 15.13	\pm 62.68	\pm 36.83	\pm 7.30

P-value with respect to normal rats, * $P < 0.05$, *** $P < 0.001$.

P-value with respect to the condition without the Russell's viper venom administration, ☆ $P < 0.05$, ☆☆ $P < 0.01$, ☆☆☆ $P < 0.001$.

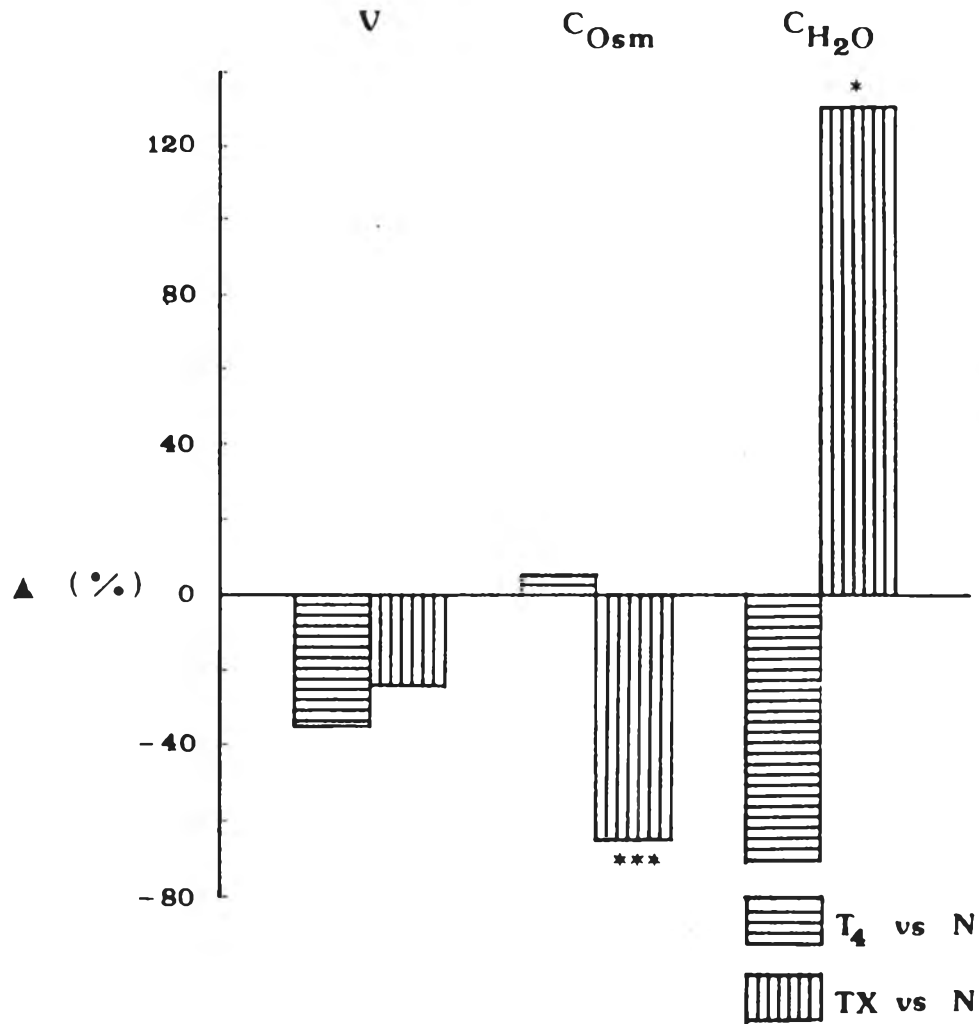


Fig. 8

Percent changes of the rate of urine flow , osmolar clearance and free water clearance in hyperthyroid and hypothyroid rats in comparison to normal rats .
 P-value with respect to normal rats , * $P < 0.05$, *** $P < 0.001$.

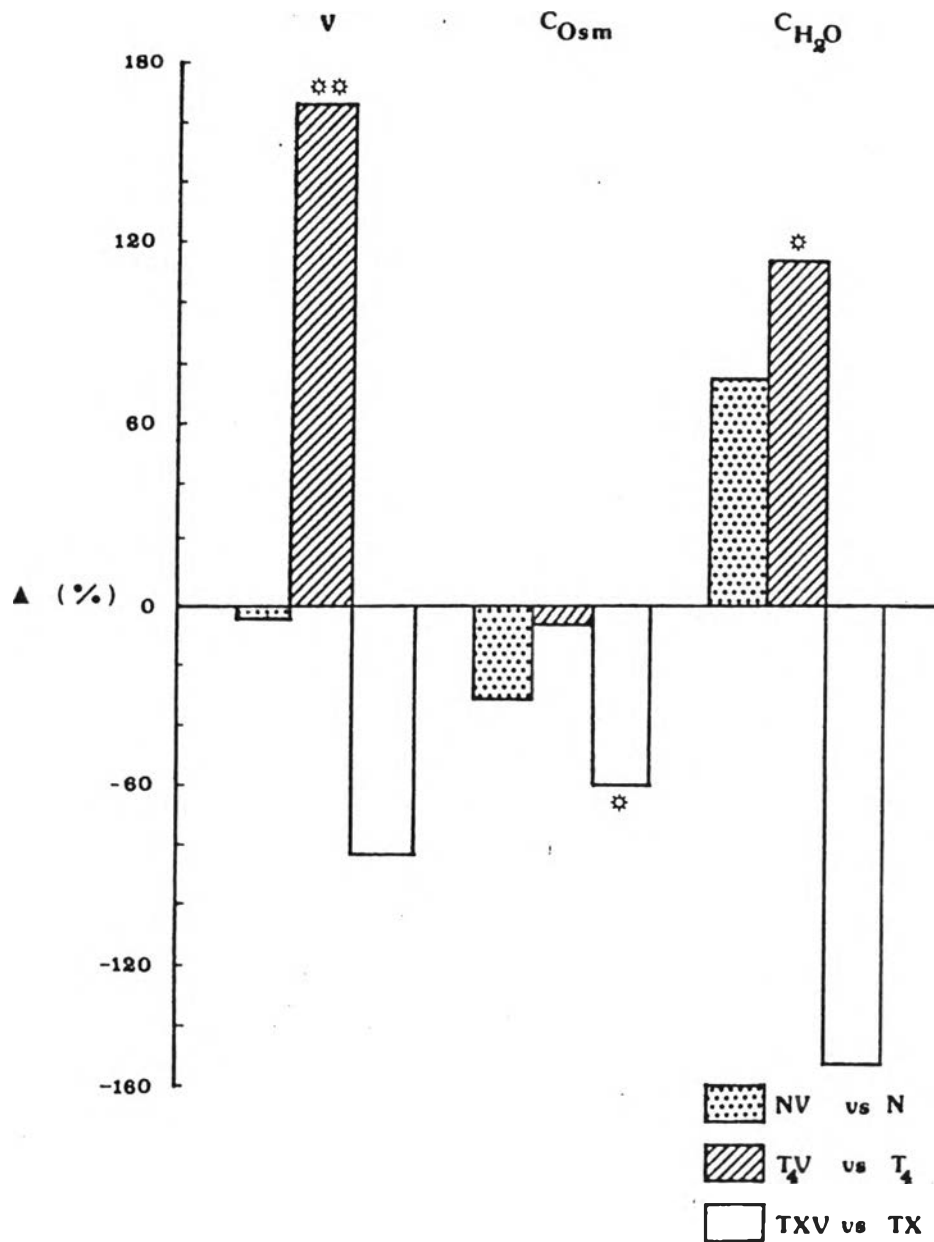


Fig. 9

Percent changes of the rate of urine flow , osmolar clearance and free water clearance in normal , hyperthyroid and hypothyroid rats with and without the Russell's viper venom administration .

P-value with respect to the condition without the Russell's viper venom administration , * $P < 0.05$, ** $P < 0.01$.

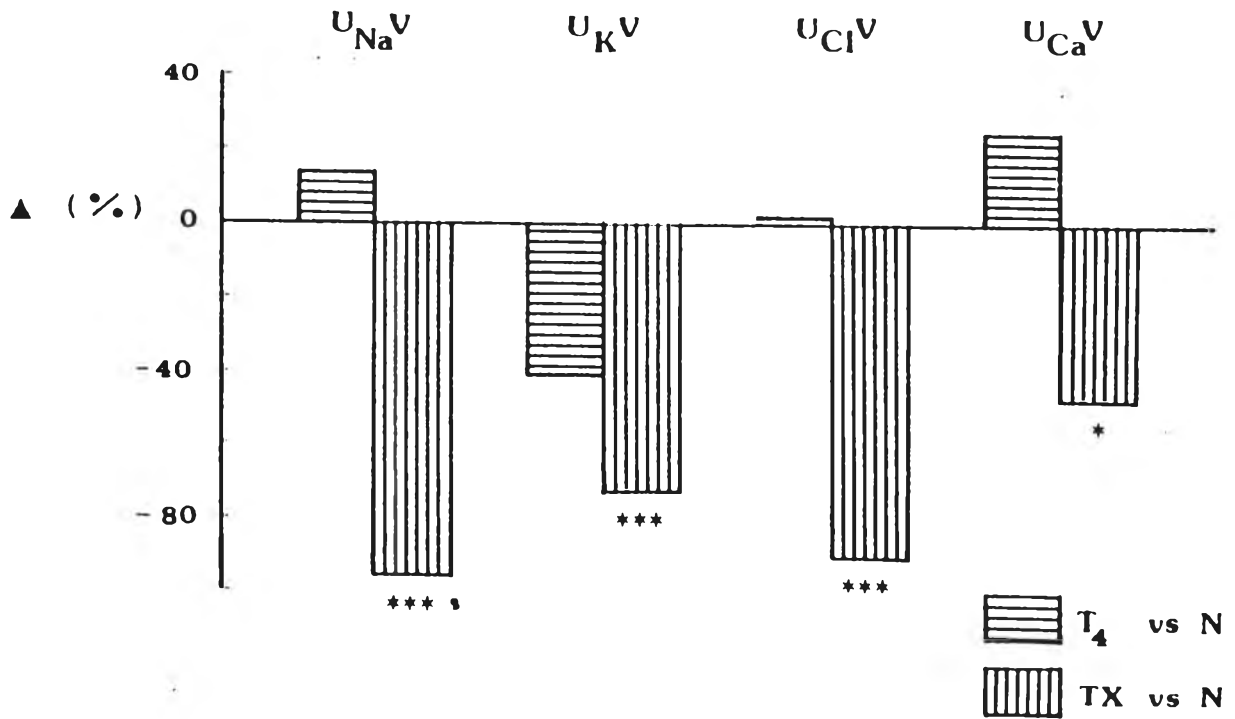


Fig. 10 Percent changes of urinary excretion of electrolytes in hyperthyroid and hypothyroid rats in comparison to normal rats .

P-value with respect to normal rats , * $P < 0.05$, *** $P < 0.001$.

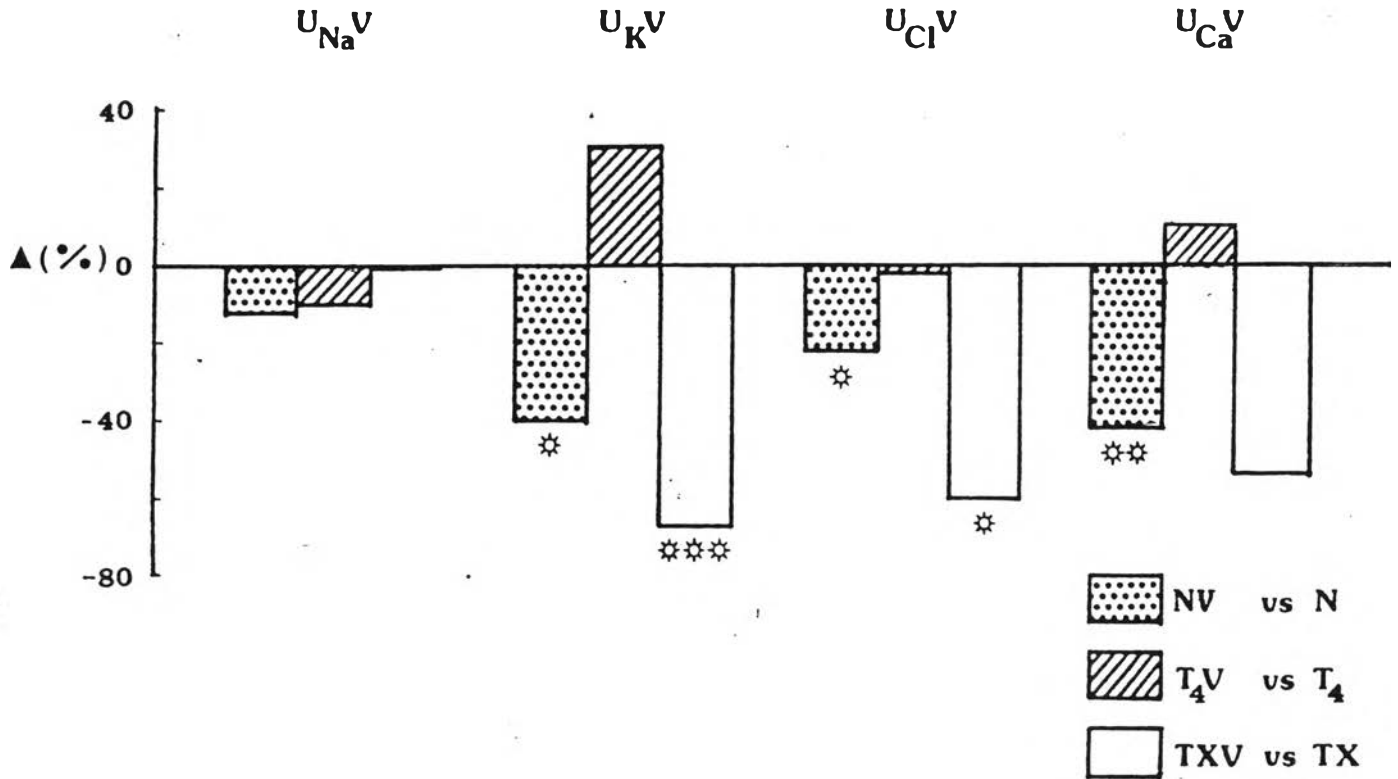


Fig. 11 Percent changes of urinary excretion of electrolytes in normal, hyperthyroid and hypothyroid rats with and without the Russell's viper venom administration. P-value with respect to the condition without the Russell's viper venom administration, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

plasma calcium (Table 5 and Fig.12).

The effect of the venom on plasma concentration of electrolytes are presented in Table 5. There were no significant differences in plasma osmolality, plasma sodium and potassium observed in all three groups of rat received the venom injection (Fig.13). Significant alterations in plasma chloride and calcium were noted only in T_4V group, increased by approximately 5% and 14% respectively from T_4 group ($p < 0.05$).

Plasma creatinine concentration (P_{Cr}) and plasma urea concentration (P_{urea}) were not affected by L-thyroxine injection but a significant difference in P_{Cr} and P_{urea} between thyroidectomized rat and intact rats were observed as shown in Table 5 and Fig.14.

Plasma creatinine concentration was affected by the venom by increased significantly in NV and TXV group (Table 5 and Fig.15), higher by approximately 63% and 33% respectively when compared to the condition without given the venom ($p < 0.05$). Plasma urea concentration was also significantly increased in NV group ($p < 0.001$) but did not alter in T_4V and TXV group.

The effect of L-thyroxine injection and thyroidectomy on fractional excretion of electrolytes are presented in Table 6 and Fig.16. The remarkable findings was a decrement in fractional excretion of sodium (FE_{Na}), potassium (FE_K) and chloride (FE_{Cl}) ($p < 0.001$) in thyroidectomized rats while fractional excretion of calcium (FE_{Ca}) increased significantly

Table 5 Effect of subcutaneous injection of Russell's viper venom on plasma osmolality, plasma concentration of electrolytes, creatinine and urea in normal (N), hyperthyroid (T₄) and hypothyroid (TX) rats. (Mean \pm S.D.)

	N	N + Venom	T ₄	T ₄ + Venom	TX	TX + Venom
P _{osm} (mOsm/kg)	281.25 \pm 7.78	280.75 \pm 10.63	276.50 \pm 7.21	277.50 \pm 6.78	267.50* \pm 13.25	267.00 \pm 13.24
P _{Na} (mEq/L)	138.13 \pm 3.91	138.25 \pm 4.83	140.38 \pm 3.25	140.00 \pm 2.27	131.75* \pm 5.56	130.83 \pm 7.83
P _K (mEq/L)	3.20 \pm 0.18	3.61 \pm 0.52	3.20 \pm 0.16	3.09 \pm 0.24	4.23*** \pm 0.52	4.27 \pm 0.45
P _{Cl} (mEq/L)	109.50 \pm 5.45	109.75 \pm 6.04	109.00 \pm 5.83	114.63* \pm 3.58	99.50** \pm 5.45	104.60 \pm 6.73
P _{Ca} (mg%)	5.86 \pm 1.62	5.89 \pm 0.48	6.17 \pm 0.76	7.05* \pm 0.35	4.91 \pm 1.29	4.90 \pm 1.07
P _{creatinine} (μ g/ml)	5.65 \pm 2.02	9.22* \pm 4.11	4.95 \pm 0.81	5.91 \pm 2.26	8.36* \pm 2.08	11.15* \pm 2.79
P _{urea} (mg%)	19.57 \pm 3.29	35.77*** \pm 9.46	20.88 \pm 3.75	25.81 \pm 6.46	30.87*** \pm 5.51	36.42 \pm 9.41

P-value with respect to normal rats, *P < 0.05, **P < 0.01, ***P < 0.001.

P-value with respect to the condition without the Russell's viper venom administration, *P < 0.05, ***P < 0.001.

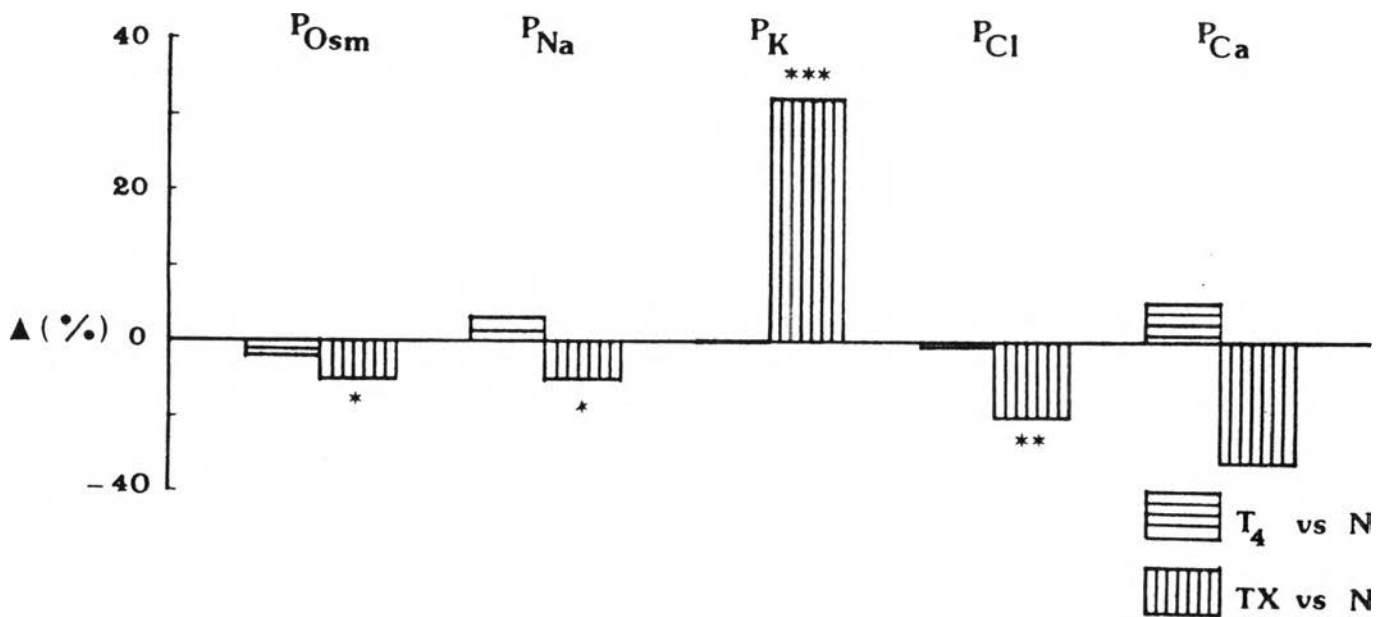


Fig. 12

Percent changes of plasma osmolality and plasma electrolytes concentration in hyperthyroid and hypothyroid rats in comparison to normal rats. P-value with respect to normal rats, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

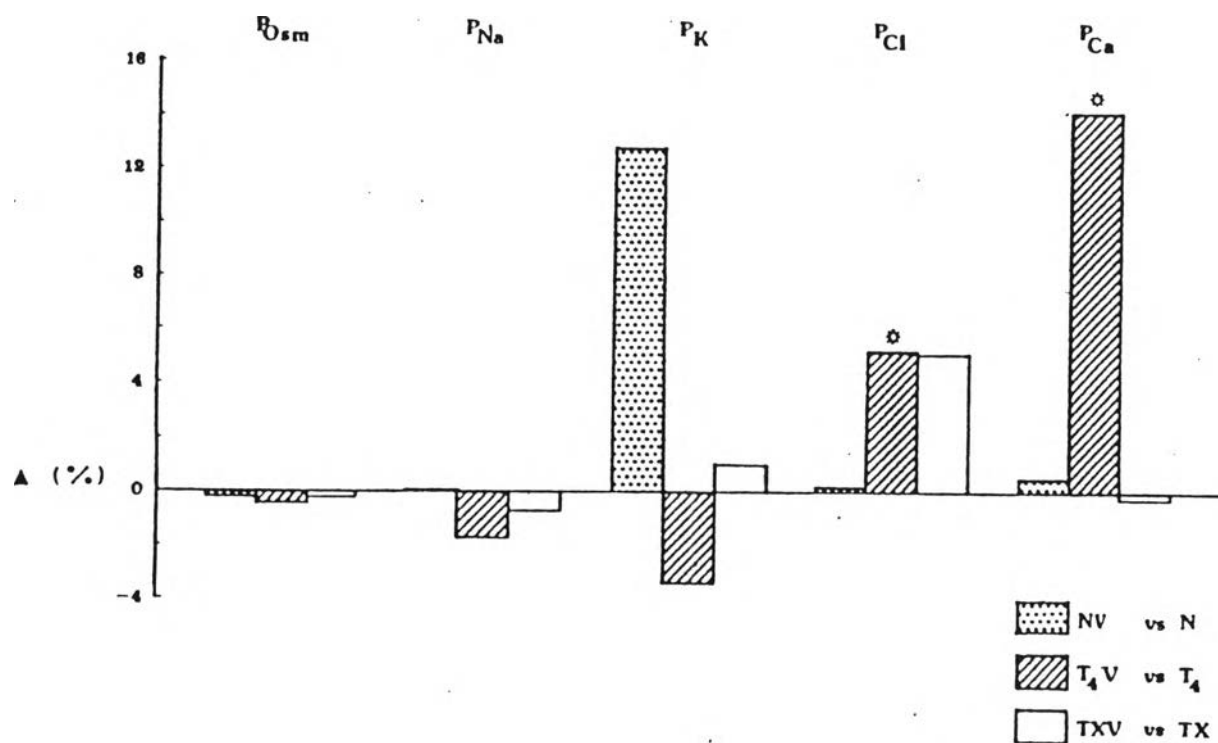


Fig. 13 Percent changes of plasma osmolality and plasma electrolytes concentration in normal, hyperthyroid and hypothyroid rats with and without the Russell's viper venom administration.

P-value with respect to the condition without the Russell's viper venom administration, * $P < 0.05$.

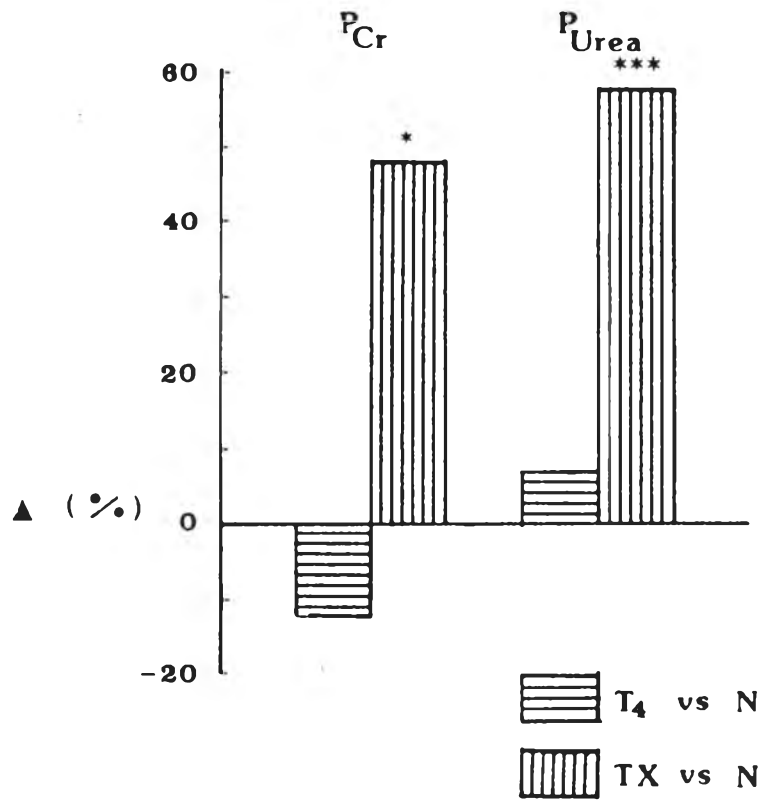


Fig. 14

Percent changes of plasma creatinine and urea concentration in hyperthyroid and hypothyroid rats in comparison to normal rats .

P-value with respect to normal rats . * $P < 0.05$, *** $P < 0.001$.

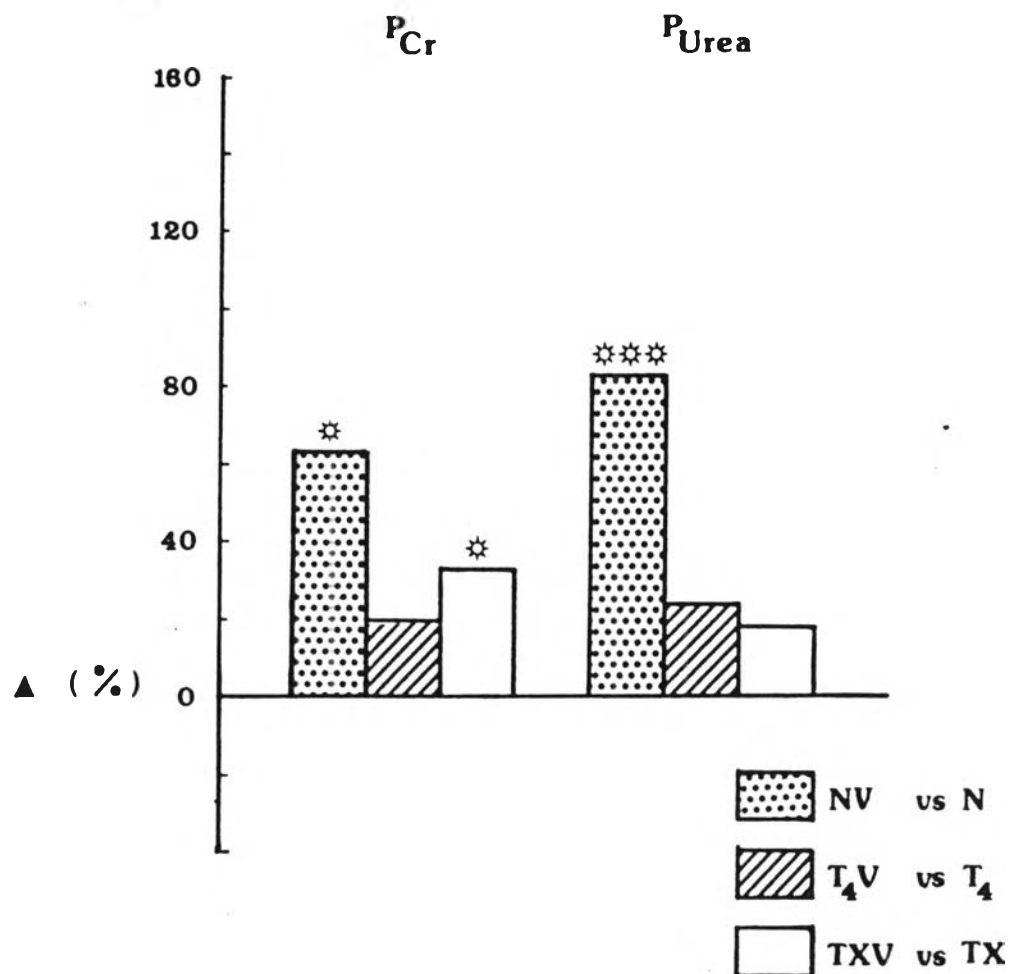


Fig. 15

Percent changes of plasma creatinine and urea concentration in normal, hyperthyroid and hypothyroid rats with and without the Russell's viper venom administration.

P-value with respect to the condition without the Russell's viper venom administration, * $P < 0.05$, *** $P < 0.001$.

Table 6 Effect of subcutaneous injection of Russell's viper venom on fractional excretion of electrolytes in normal (N), hyperthyroid (T_4) and hypothyroid (TX) rats. (Mean \pm S.D.)

	N	N + Venom	T_4	T_4 + Venom	TX	TX + Venom
FE_{Na}	1.28	2.75 ^{☼☼}	1.68	1.26	0.14 ^{***}	0.81 ^{☼☼☼}
(%)	± 0.62	± 0.93	± 0.60	± 0.53	± 0.03	± 0.40
FE_K	33.41	45.46 [☼]	25.13	32.92	19.20 ^{***}	46.62 ^{☼☼☼}
(%)	± 8.00	± 13.53	± 12.44	± 12.02	± 5.28	± 14.09
FE_{Cl}	2.19	4.24 ^{☼☼}	2.76	2.06	0.56 ^{***}	1.83 ^{☼☼☼}
(%)	± 0.66	± 1.28	± 1.10	± 1.05	± 0.22	± 0.61
FE_{Ca}	0.71	1.08 ^{☼☼}	0.98 ^{**}	0.76	1.14 ^{**}	5.07 ^{☼☼☼}
(%)	± 0.17	± 0.29	± 0.14	± 0.35	± 0.37	± 2.53

P-value with respect to normal rats, ** $P < 0.01$, *** $P < 0.001$.

P-value with respect to the condition without the Russell's viper venom administration, ☼ $P < 0.05$, ☼☼ $P < 0.01$, ☼☼☼ $P < 0.001$.

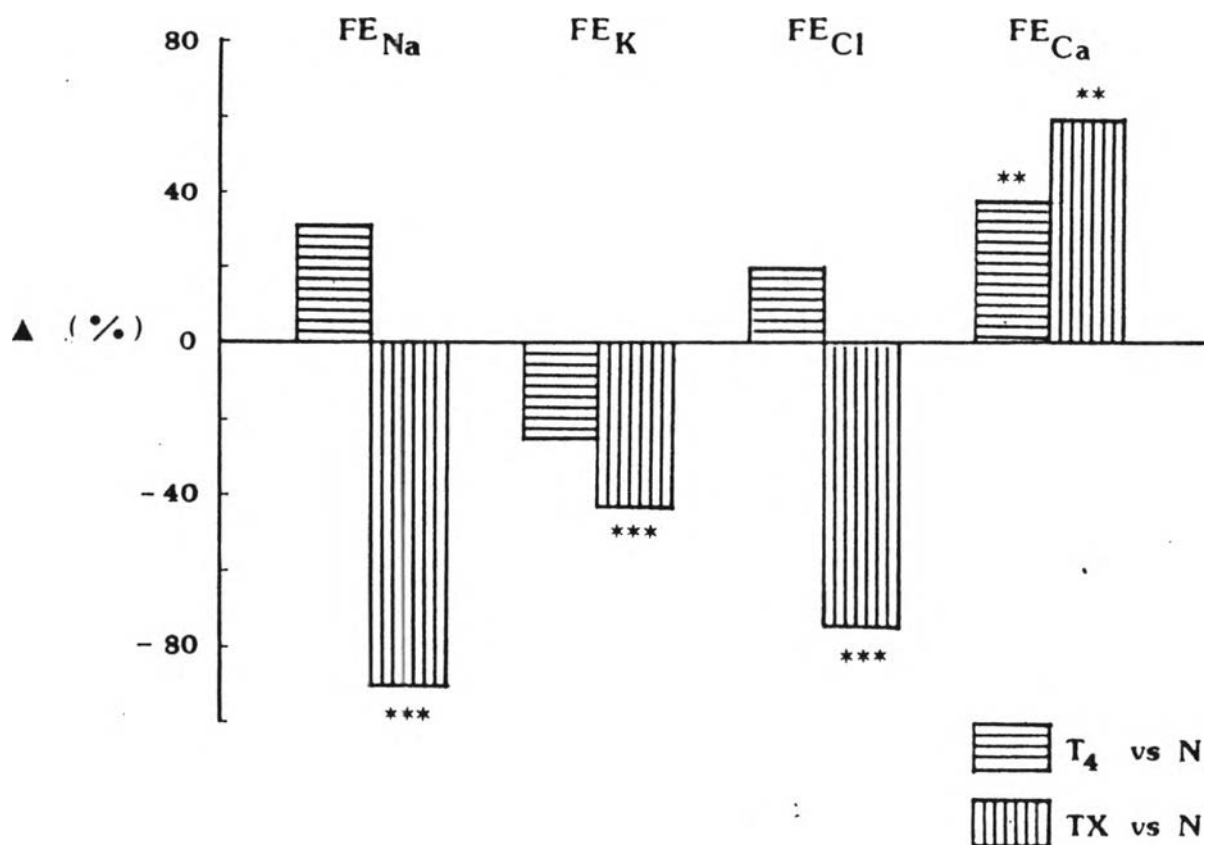


Fig. 16 Percent changes of fractional excretion of electrolytes in hyperthyroid and hypothyroid rats in comparison to normal rats .

P-value with respect to normal rats , ** $P < 0.01$, *** $P < 0.001$.

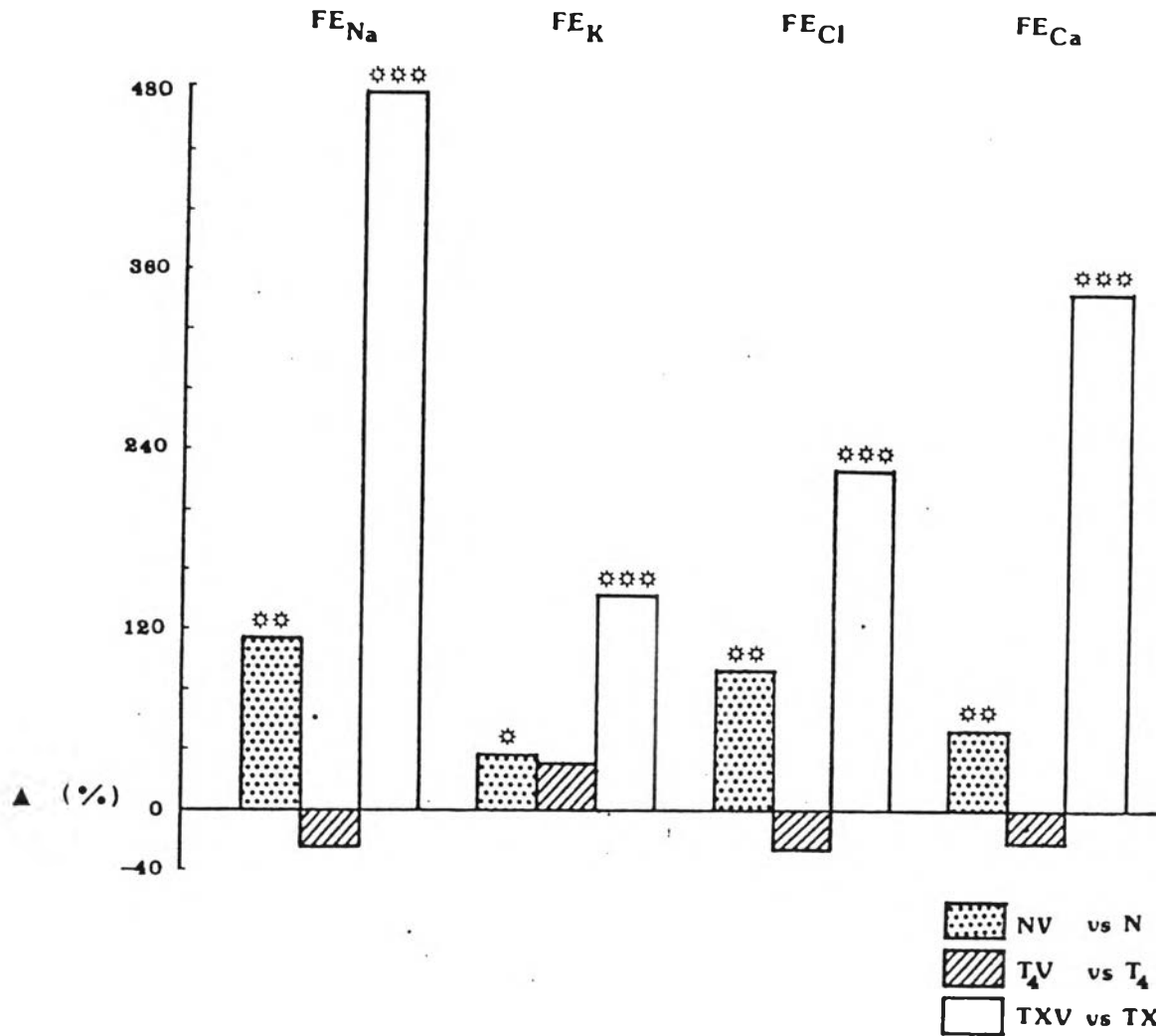


Fig. 17. Percent changes of fractional excretion of electrolytes in normal, hyperthyroid and hypothyroid rats with and without the Russell's viper venom administration.

P-value with respect to the condition without the Russell's viper venom administration, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

in both hyperthyroid and hypothyroid rats and no significant change in fractional excretion of sodium, potassium and chloride were recorded in hyperthyroid rats.

Subcutaneous injection of the venom caused a significant increase in fractional excretion of all electrolytes in normal and in thyroidectomized rats (Table 6 and Fig.17). While no significant changes in fractional excretion of all electrolytes were noted in hyperthyroid rats.

Changes in renal pathology in normal, hyperthyroid and hypothyroid rats given Russell's viper venom.

Pathological study of kidneys from saline-injected normal rats showed a mild degree of hyalin casts in which hemoglobin could be demonstrable by Puchtler's method (Luna,1968) in the lumina of proximal and distal convoluted tubules of cortical area. In kidneys of normal rats given Russell's viper venom injection both renal cortex and medulla showed moderate degree of hemoglobin casts, hyalin droplets degeneration in epithelial cells and focal hemorrhages in the intertubular area. In thyroidectomized rats given normal saline injection, a mild degree of focal hemorrhagic lesion was observed while thyroidectomized rats given the venom injection revealed both focal hemorrhagic lesion and a high degree of hemoglobin casts and hyalin droplets degeneration in epithelial cells of both renal cortex and medulla. In addition, a focal thrombophlebitis was presented in this group. No evidence of hemoglobin casts and

focal hemorrhages were observed in hyperthyroid rats given normal saline injection, while in hyperthyroid rats given the venom injection, a mild degree of hemoglobin casts were found in both cortex and medulla.

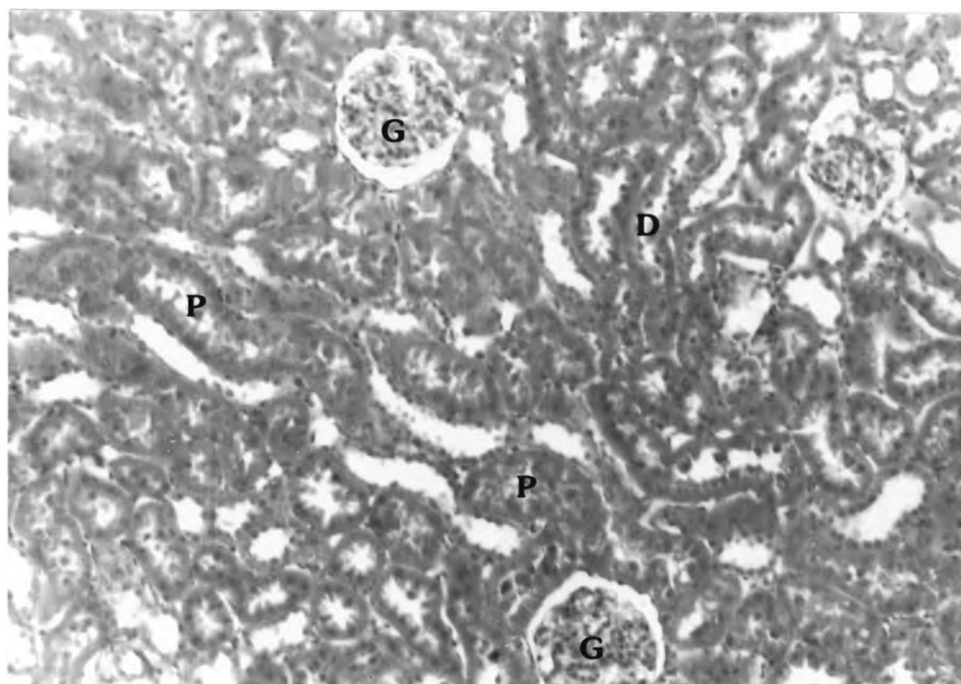


Fig. 18 Microphotograph of glomeruli (G) , proximal (P) and distal (D) convoluted tubules of normal rats.
(H & E 10X10)

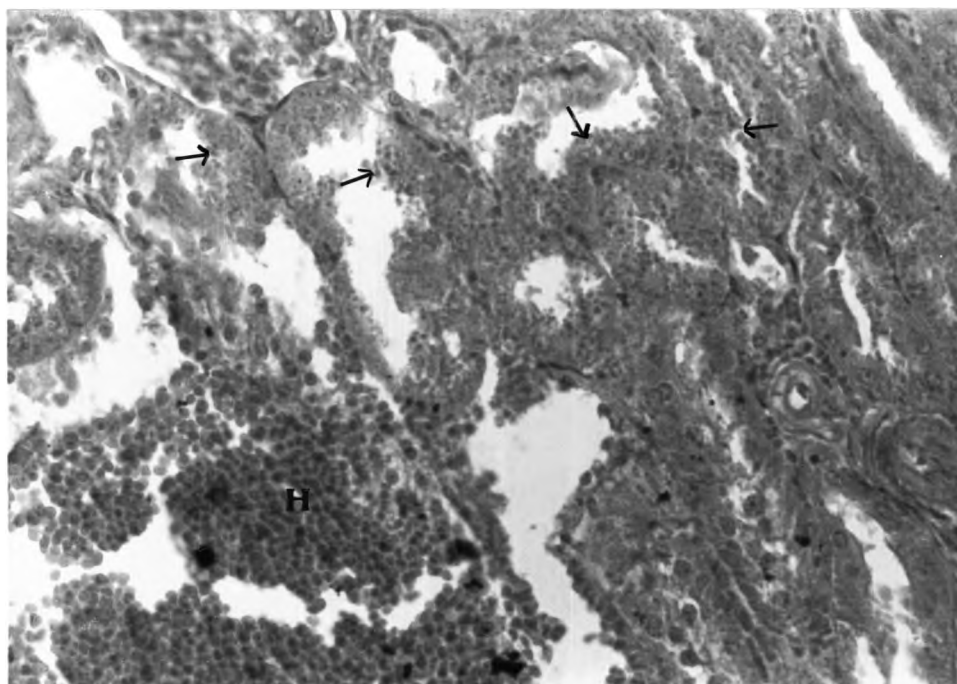


Fig. 19 Focal hemorrhage (H) in intertubular area and hyaline droplets (arrows) in epithelial cells of normal and thyroidectomized rats received the Russell's viper venom injection. (H & E 20X10)

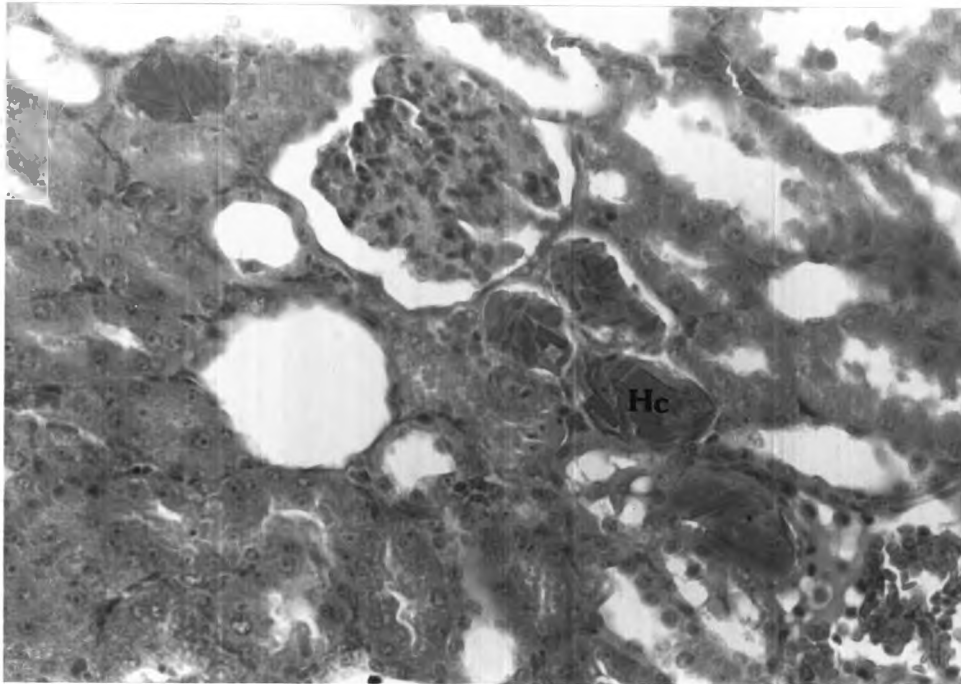


Fig. 20 Hyalin casts (Hc) in lumen of convoluted tubule of normal and thyroidectomized rats received the Russell's viper venom injection. (H & E 20X10)

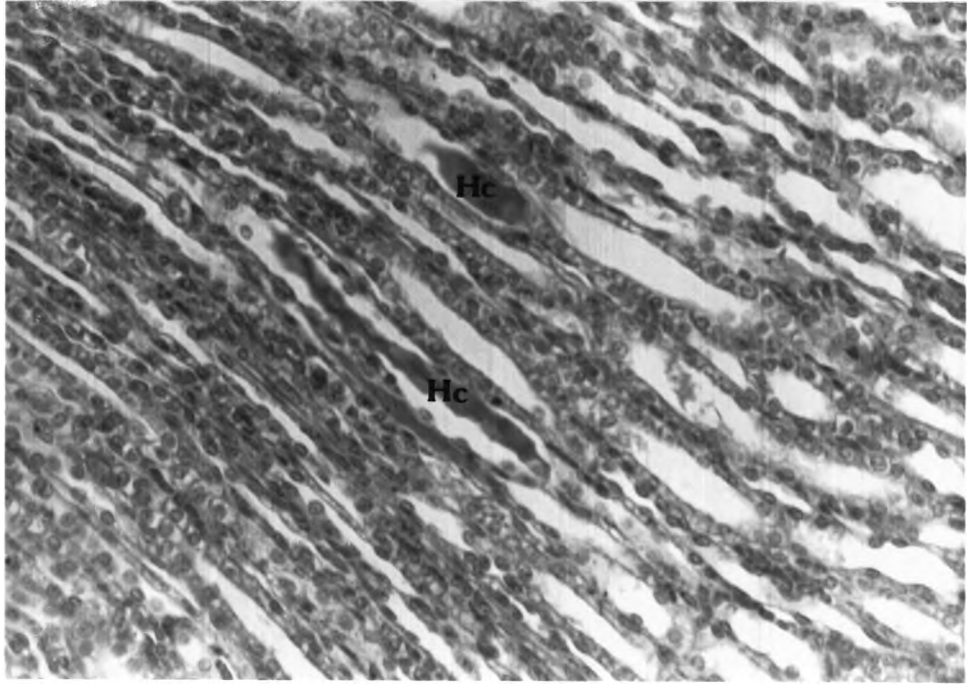


Fig. 21 Hyalin casts (Hc) in lumen of Henle's loop and collecting tubule in medulla of normal and thyroidectomized rats received the Russell's viper venom injection. (H & E 20X10)

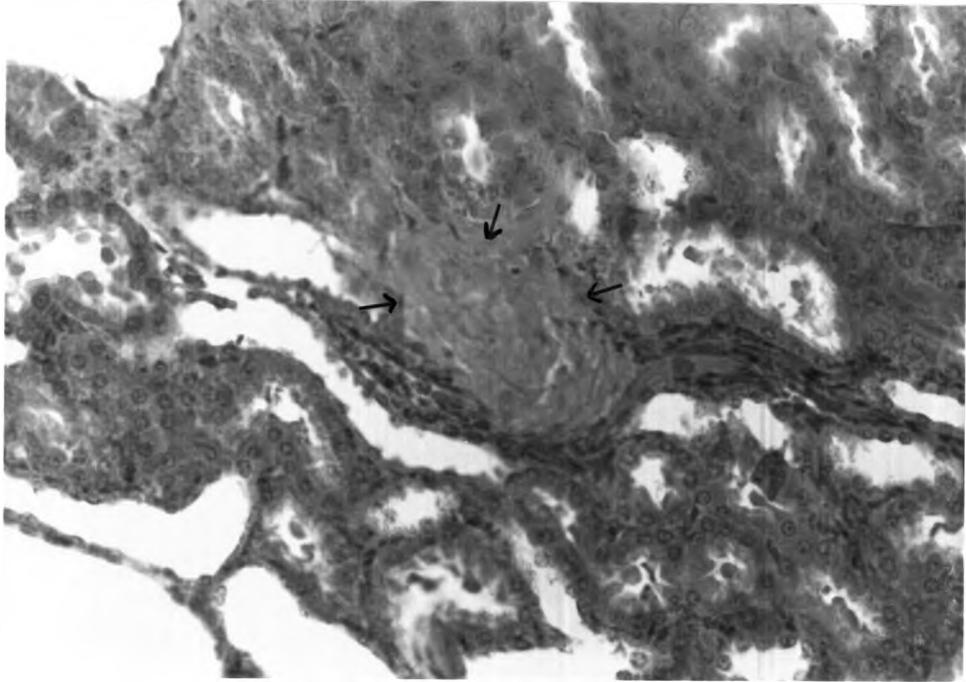


Fig. 22 Focal thrombophlebitis (arrows) in thyroidectomized rats received the Russell's viper venom injection.
(H & E 20X10)

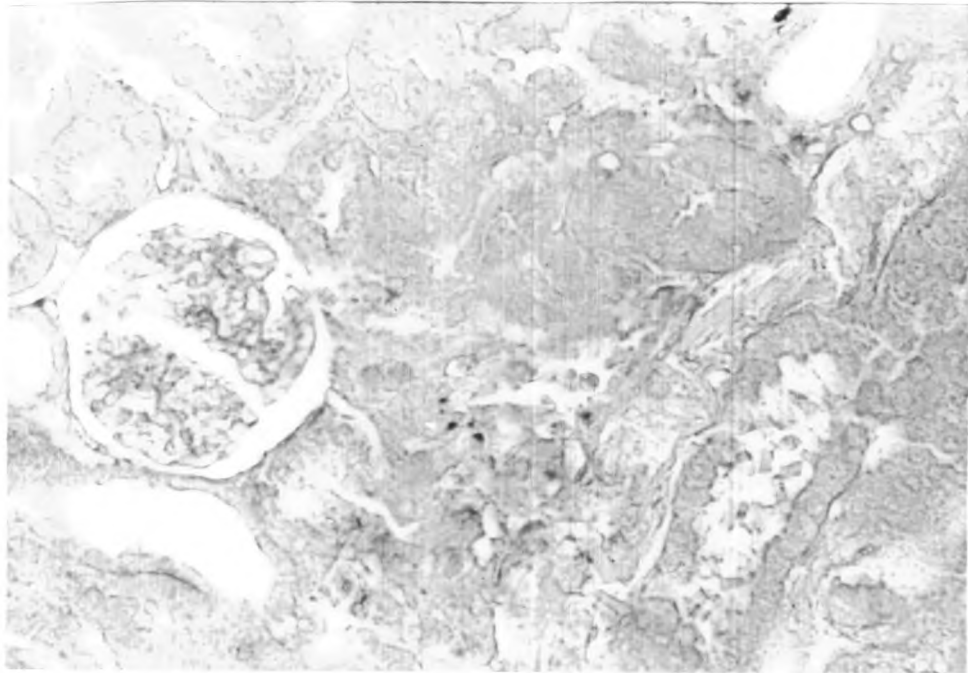
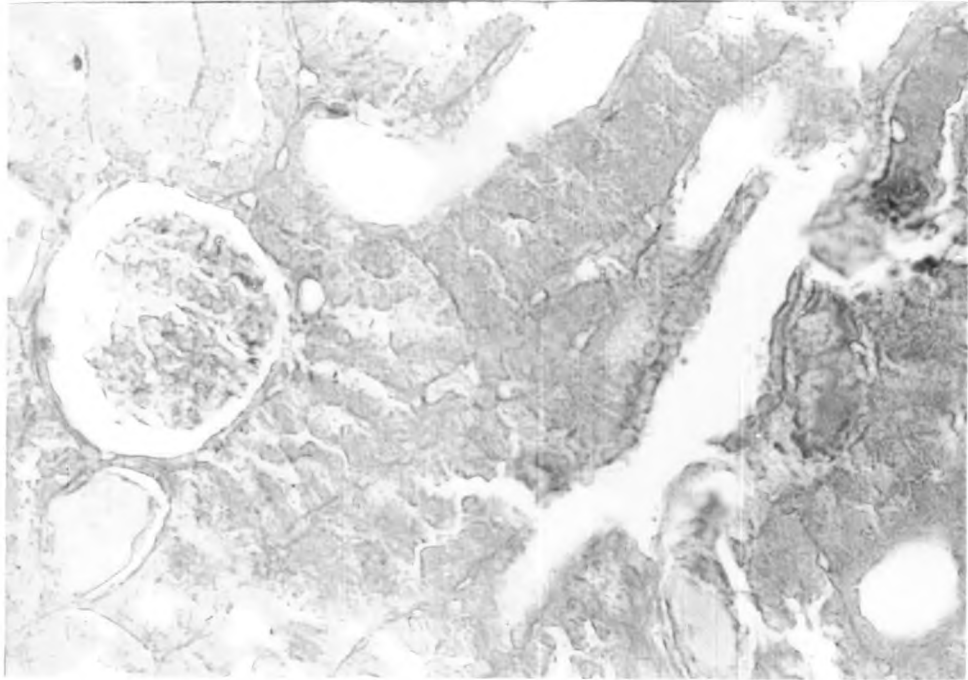


Fig. 23 Hyaline casts in which greenish stained hemoglobin could be demonstrable by Puchtler's method. (20x10)