CHAPTER V

DISCUSSION

The present investigation is designed to study the direct effects of Russell's viper venom on renal hemodynamics and histopathological changes. The dose of Russell's viper venom 0.05 mg/kg bw. and a higher dose of 0.1 mg/kg bw. which have been exactly studied from the preliminary study were used for investigating here.

In our results, there were obviously gradual decreases in mean arterial blood pressure and renal arterial blood pressure following both different doses of Russell's viper venom infusion. Heart rate was suddenly reduced over five to fifteen minutes after venom infusion. These results are similar to the other workers (Vick *et al.*, 1967; Tangthanathanich, 1983; Tongvongchai, 1984). In the past decade, many investigators have studied the cardiovascular effects of Russell's viper venom. In 1934, Chopra and Chowhan have postulated that the hypotensive effect of Russell's viper venom might probably due to the local dilatation of the capillaries of splanchnic area. An evisceration preceding venom infusion can prevent the initial phase of hypotension and bradycardia in intact animals so that Russell's viper venom maybe cause vasodilatation of the capillaries in the pooling of the blood in the hepato-splanchnic bed (Vick *et al.*, 1967). However, vagotomy cannot prevent the sharp fall in mean arterial blood pressure exception bradycardia (Vick *et al.*, 1967). Generally, it has been known that

heart rate varies inversely with the arterial blood pressure (Marey, 1859). It has been firmly established that changes in heart rate are mediated reflexly, the afferent arm of the reflex originating in the baroreceptors locate in the carotid sinus and aortic arch (Winder, 1937; Heymans and Laden, 1924). The hypotensive action of Russell's viper venom may not relate with reflex impulse and also the nervous centers are not much affected, since the same results are obtained in decerebrated animals (Chopra and Chowhan, 1934; Lee, 1948). Capillaries paralysis following venom perfusion has been observed similar to that of histamine induced hypotension (Iswariah and David, 1932). The fall in blood pressure may due to dilatation of the capillaries although the arterioles are cetually contracted (Chopra and Chowhan, 1934; Lee, 1948). It has been demonstrated that histamine produces a profound decrease in blood pressure by dilatetion of the capillaries of the splanchnic area and not of the arteries or arterioles (Iswariah and David, 1932). In addition to, Lee (1944, 1948) has attributed that diminution of arterial blood pressure seems due to peripheral action and intravascular clotting effect. In the isolated heart of frogs and rabbits, the venom induces a stimulant action in small dose but an inhibitory action in the large dose (Lee, 1948). The direct effect of Russell's viper venom is on netther myocardium nor the nervous apparatus of the heart (Iswariah and David, 1932; Chopra and Chowhan, 1934). Total peripheral resistance and cardiac output are also decreased significantly in the second hour period, although mean arterial blood pressure is recurred to the control level (Tongvongchai, 1984). Therefore, the initial phase of envenomation probably causes a decrease in venous return and contributes to the low cardiac output concomitantly with decrease in mean arterial blood pressure (Tongvongchai,

1984). Futhermore, the phospholipase A_2 from Russell's viper venom has been shown to have many actions such as hypotension (Ho and Lee, 1981; Huang, 1984), neurotoxicity (Lee and Ho, 1982), local capillary damage and tissue necrosis (Suzuki and Iwanaga, 1970), and anticoagulant action (Boffa *et al.*, 1982). Recently, phospholipase A_2 or subfraction of phospholipase A_2 has produced an increase in the lung perfusion pressure accompanies with a release of prostaglandins - like and leukotriene-like substances and also histamine (Huang, 1984a). These autacoids releasing from lung increase vascular permeability and maybe affected in pulmonary edema formation. In addition to the clinical report, pulmonary edema and circulatory failure have been observed in the victims (Jeyarajah, 1984). An increase in lung perfusion pressure which maybe restrict to blood return to the heart, leads to a decrease in cardiac output and indirectly induces a greater hypotensive effect (Huang 1984b). Moreover, the part of hypotensive action of phospholipase A_2 fractions maybe due to the actions of histamine, prostacyclin and leukotrien releasing (Huang, 1984b). In this viewpoint, Sket and Gubentek (1976) have studied the hypotensive effect from phospholipase A_2 fractions and crude venom of *Vipera ammodytes*, using indomethacin as an inhibitor of prostaglandin synthesis and emphasize that indomethacin (50 mg/kg bw.) dose not abolish a fall in systemic arterial blood pressure after administration of phospholipase A_2 or crude venom. They have suggested the existence of at least two different pharmacological effects of phospholipase A_2 which one can be blocked by indomethacin and the other which is restricted to its action. However, cardiovascular effects of Russell's viper venom in the indomethacin - pretreated dogs have been shown a less reduction in mean arterial blood pressure and recovers to the

control level in a short period as compares to the control group which non indomethacin-pretreat whereas heart rate is variable response in both groups (Tongvongchai, 1984). Chaiyabutr *et al.* (1984) have attributed that the catecholamine and other hormones involvenment maybe release in the compensatory period because of no this period reveals to recover following administration of converting enzyme inhibitor (MK-422 or Enalapril maleate) in either pre or post envenomation. At about this period, they have emphasized that the renin-angiotensin activity is being the one main mechanism. On the other hand, it is generally accepted that Russell's viper venom consists of kinin-releasing agent and factor Xa amongst many other enzymes, principally affect the cardiovascular and haemostatic mechanisms (Macfarlane, 1964; 1965; Marsh and Whaler, 1978; Esnouf 1982). Following experimental injection of many crude venom which containing phospholipase and kinin releasing agent, both systolic and diastolic blood pressure diminish precipitously giving a marked rise in pulse pressure (Whaler, 1975). Inasmuch as the cardiac output appears at this time to be little affected, the major action is on peripheral resistance, perhaps mediated by bradykinin (Marsh and Whaler, 1978). Nevertheless, the direct evidence for the participation of this peptide is sparse. Powerful though the kinins maybe, their effects are essentially short acting and do not account for the subsequent hypotension and perhaps ultimate death. In our observations, the higher dose of Russell's viper venom (0.2 - 0.5 mg/kg bw.) produced precipitously profound fall in arterial blood pressure which be unable to rerive to the control level. Furthermore, the highest dose (1.0 mg/kg bw.) caused a serious diminution of arterial blood pressure associated with abrupt ceasation of respiration. We attribute the dose-dependent of Russell's viper venom

affects on the gradual arterial blood pressure changes. In this investigation, the initial hypotension occurred within 20-30 minutes associated with reduction of heart rate. It is noteworthy either arterial blood pressure or heart rate is declined together, thus these may suggest that the alteration of heart rate is indirectly effect so.

It will be observed that packed cell volume was reduced significantly following Russell's viper venom 0.1 mg/kg bw. infusion whereas it tended to be high in half an hour and then followed to be low but not achieve a statistical significance in venom 0.05 mg/kg bw. infusion group. In both group, hematuria or hemoglobinuria was appeared within 1-2 hour infusion. Frank hemoglobinuria has been obviously observed either in the victims (Reid, 1968; Peris *et al.*, 1969) or in rats (Thein *et al.*, 1985). Condrea in 1979 has suggested that intravascular hemolysis could be represented by the degree of hemoglobinuria. It is generally accepted that Russell's viper venom has been known as a powerful coagulant property and be able to produce homeostatic abnormality. According to factor Xa containing in Russell's viper venom, intravascular coagulation produces a characteristic subsequence of changes in components of the homeostatic mechanism (McKay and Margaretten, 1967; Denson, 1969; Marsh and Whalter, 1978) and decrease in platelets, circulating fibrinogen, prothrombin complex, factors V, VII, VIII and X so that these changes lead to further diminution of the concentration of fibrinogen and other factors. Intravascular coagulation and fibrinolysin activation usually occur simultaneously (McKay and Margaretten, 1967, Denson 1970), hence, disseminated intravascular coagulation causes intravascular hemolysis (Brain *et al.*, 1967). Moreover, the Russell's viper venom damages the blood vessels

especially to the arterioles and capillaries (Efrati and Reif, 1953) and this consistents with a widespread capillaries endothelial damage by increasing capillary permeability and mediated through the release of histamine and 5-HT (Fearn *et al.*, 1964). Recently, Vishwanath *et al.* (1985) have documented the phospholipase A_2 causes necrosis and hemorrhage in liver of mice. This evidences advocate the prediposing factors include intravascular coagulation, intravascular hemolysis and hemorrhagic activity of Russell's viper venom exhibite a decrease in packed cell volume and might be indirect partly lead to hypotensive effect.

We could not eliminate the extra-renal factors that influence upon the renal function and histopathological changes so that remarkable two patterns were firstly, initial hypotension and secondly, the compensatory period have been considered. During inital hypotension, effective renal plasma flow, renal blood flow and glomerular filtration rate were diminished significantly following both different doses of venom infusion. On the other hand, as the arterial blood pressure was recovered to the control level, the effective renal plasma flow, renal blood flow and glomerular filtration rate were maintained at a lower level. An increase in renal vascular resistance of both Russell's viper venom 0.05 mg/kg bw. and 0.1 mg/kg bw. following initial venom infusion was also exhibited prominently at the end of experiment. Effective renal plasma flow was diminished proportionally with reduction of glomerular filtration rate so that the filtration fraction was maintained reasonably well. An initial reduction in arterial blood pressure may produce restriction to blood flow to both of kidneys in order to conserve a large amount of blood flow to the essential

organs such as brain, etc. Futhermore, both kidneys are the primary organ that are responsible to regulate the body fluid (Ganong, 1980). A prolonged increase in renal vascular resistance associated with a marked reduction of glomerular filtration rate could induce the diminution in the urine flow rate. It is indicated that the persistent renal vasoconstriction in both kidneys has been occurred. It is interesting that renal vascular resistance increases persistently although the arterial blood pressure is recovered to the control level. Chaiyabutr *et al.* in 1984 have described that this mechanism is probably due to the activities of renin-releasing and other hormones involvement in the compensatory period. In our opinion, the initial hypotension associates with a marked reduction of effective renal plasma flow, renal blood flow and glomerular filtration rate and increment of renal vascular resistance are sufficient advocacy that postulate the intrarenal baroreceptor of renal sympathetic nerve is activated and releases renin secretion since the juxtaglomerular apparatus is innervated by sympathetic nerve (Levens *et al.*, 1981). Hence, an increase in ability of renin-angiotensin system has been implicated as a cause of rise in renal vascular resistance associated with remarked reduction of renal blood flow and glomerular filtration rate. According to the other hormone including kinin, prostaglandin, histamine, 5-HT, leukotriene and the abnormalities of blood coagulation, the vascular effects maybe involve as the factors response to modulate the renal hemodynamic changes following venom infusion. The fall in glomerular filtration rate produces a diminution of filtered load of electrolytes and urine flow rate. Nonetheless, urine flow rate is not indicated to show a statistically significant value, since be differently responsive time of individual animals.

The plasma creatinine of both different dose of venom infusion groups are not increased significantly but tended to be high especially in the venom 0.1 mg/kg bw. infusion group. However, blood urea concentrations are more increased than the control period of each venom infusion group. Dehydration validity could be abolished since infusion of isotonic saline solution during the experimental period. In acute renal failare (ARF), azotemia has been appeared in one or more weeks' duration in man (Bull *et al.*, 1950; Myer *et al.*, 1982) and at least 48 hours in uranyl nitrate induced acute renal failure (ARF) in dogs (Stein *et al.*, 1975). In these results may indicate the secretory tubular dysfunction for the waste product as usual (Anderson *et al.*, 1977). It is generally finding that the plasma concentration of urea, creatinine, K^+ and H^+ increase in ARF (Minuth *et al.*, 1976; Anderson *et al.*, 1977). In the Russell's viper venom 0.05 mg/kg bw. infusion group, the plasma concentration of K^+ is also increased significantly at the fifth hour period when compared with the mean control value whereas the plasma of Na^+ and Cl^- are not affected. Moreover, in the large dose of venom 0.1 mg/kg bw., the plasma concentrations of potassium and chloride are elevated significantly. It is reasonable to recognize the obvious validity occurs probably due to ARF since oliguria appears following a marked reduction of glomerular filtration during acidosis, affects to increase in the waste product.

Normally, renal tubles can remove many substances from glomerular filtrate; also, in some instances other substances are added to the tubular fluid. Renal tubular function are therefore reabsorption and secretion of fluid and solute (Dousa, 1978). In our results, an increase in fractional excretion of sodium is not clearly

indicated a decrease in tubular reabsorption function, which seems due to the renal tubular cell damage. Since the fractional excretion of sodium is also elevated during isotonic saline infusion, possibly responsive saline diuresis in the saline control group. Besides an increase in fractional excretion of sodium, the fractional chloride and potassium excretion as well as the urinary excretion rate of electrolytes are also elevated too. A variety of evidence is consistented with the concept that small saline load in the dogs results in a net decrease in tubular reabsorption of sodium, osmotically active substances, and finally producing natriuresis (De Wardener *et al.*, 1961; Dirks *et al.*, 1965; Earley and Friedler, 1964; 1965a). Moreover, a net decrease in tubular reabsorption of sodium as well as natriuresis are independented on a decline in glomerular filtration rate (De Wardener *et al.*, 1961; Earley and Friedler, 1965 a; Dirks *et al.*, 1965; Rector *et al.*, 1966, 1967), filtered load of sodium (De Wardener *et al.*, 1961), angiotensin and aldosterone (Levinsky and Lalone, 1963), vagotomy, carotid denervation and upper thoracic sympathectomy (Mills *et al.*, 1961). Many laboratories have demonstrated hemodynamic and physical factors can alter the tubular reabsorption and excretion of sodium (Earley and Friedler, 1965 a, 1965 b, 1966). The factors responsible saline diuresis remains unknown, although the mechanism of decrease in sodium tubular reabsorption and natriuresis are still unclear. In the saline control group, the urine flow rate, together with urinary excretion of electrolytes are increased whereas the systemic blood pressure, heart rate and packed cell volume are not altered. Whether the plasma concentrations of electrolytes are not changed significantly, rendered from extracellular fluid expansion, the regulatory

tubuloglomerular system is also effectively. Generally the fractional excretion of sodium test is accepted as one important indicators for evaluating development of ARF (Espinel, 1976), since it is hardly differentiate prerenal azotemia (functional renal insufficiency) from acute tubular necrosis (vasomotor nephropathy). In the oliguric phase of each of two conditions, the renal tubule handles sodium in distinctly opposing fashions. In prerenal azotemia, the renal tubule avidly reabsorbs filtered sodium (Merrill, 1971). On the contrary, reabsorption of sodium is restricted in acute tubular necrosis (ATN) (Bull *et al.*, 1950) so that a simple test of fractional sodium excretion precisely defines tubular handling of sodium and, thus differentiates ATN from prerenal azotemia (Espinel, 1976). In ARF, an alterations in tubular functions are reflected in the fractional sodium excretion, since in the prerenal azotemia, decrease in renal blood flow and glomerular filtration rate lead to an increase in an absolute and fractional tubular reabsorption of sodium, thus the fractional sodium excretion is low (Merrill, 1971). Ordinarily, in the patients whom become prerenal azotemia have the fractional excretion of sodium less than 1 (Espinel, 1976). In controversy, the functioning nephron units excrete a large fraction of filtered sodium so that a high fractional excretion of sodium is the characteristic of ATN (Bull *et al.*, 1950). According to the clinical reports, the patients with ATN have usually fractional sodium excretion of more than 3 (Espinel, 1976). Inhibition of sodium reabsorption by diuresis also results in a high fractional sodium excretion as described in the saline control group, hence, the events must be carefully taken into consideration when fractional excretion of sodium is used to diagnose ARF. On the other hand, its disagreement with Espinel, Pru and Kjellstrand (1980; 1984) have

suggested that no difference in false-positive or negative tests or numerical results of fractional excretion of sodium. Nonetheless, Espinel *et al.*(1980) concluded that in ARF caused by shock, or spontaneous rise in fractional excretion of sodium from <1 to > 1, without concomitant increase in glomerular filtration rate, indicated the critical point at which ATN developed. Predicting renal failure indicators such as osmolar clearance and free water clearance are suggested to determine since in osmolar clearance and free water clearance are useful in predicting the onset of renal failure before the development of the oliguric state (Brown *et al.*, 1980). Some investigators have suggested that U/P osm emerged as a valuable and practical prognosticator of renal injury at the time of shock (Jones and Weil, 1971). However, the fractional sodium excretion as a predicting differentiated indicator of renal failure is significantly more increased in the Russell's viper venom 0.1 mg/kg bw. infusion group than the saline control group as shown in figure 21. It is a sufficient reason to suggest that Russell's viper venom produces ATN, since the failure of glomerular filtration accompanied by a decrease in tubular reabsorption. There are two causes of ATN, firstly, is from rapid prolonged ischemia and secondly, is from a nephrotoxic agent such as uranyl nitrate, gentamicin (Bull *et al.*, 1950; Stein *et al.*, 1980). In ischemic or anoxic cause of renal failure, the reversibility of tubular cell could occurred after restoration of oxygen and / or substrate (Bull *et al.*, 1950; Trump *et al.*, 1974). Generally, there are two systems which a particular etiologic agent be involved and produced ATN, firstly, ATP production or bionergics system including an oxidative phosphorylation by mitochondria and glycolytic system and secondly, the cell membrane including both

transport function and function as a permeability barrier between the cell interior and the extracellular space (Trump and Mergner, 1974). Recently, Russell's viper venom has been demonstrated to produce an increase in the rate of lipid peroxidation in the kidneys of mice (Ali *et al.*, 1981). In addition to, subcellular membrane damage has been related to lipid peroxidation (Tappel, 1970). Besides an increase in fractional excretion of sodium, fraction chloride and potassium excretion are also elevated simultaneously whereas filtered load of electrolytes slightly reduced. The urinary excretion rate of sodium is increased with respected to the control period of Russell's viper venom 0.1 mg/kg bw. infusion group, since fractional sodium excretion is significantly more rised than the saline control group. The decline in filtered load is affected from the failure of glomerular filtration, so that the urinary excretion rates of electrolytes are reduced.
To determine the tubular integrity, the osmolar clearance and urine flow rate are increased obviously whereas the tubular water reabsorption be fallen as shown in our data during saline diuresis. Nevertheless, free water clearance is more reduced while U/P osm. is more increased than the mean control periods of both 0.05 mg/kg bw. and 0.1 mg/kg bw. venom infusion groups. These indicate the tubular integrity is still normal, however, the urine flow rate is persistently reduced. It is noteworthy that a persistent increase in renal vascular resistance associated with a fall in glomerular filtration rate and normal tubular integrity indicate the ischemic appearance is more suspiciously than a nephrotoxicity. On the other hand, normal tubular integrity activity is also possibly meaned the rest of non-damaging nephrons are still normal function and this be only remarkable rationalization could not be excluded. Normally, the patients whom become prerenal azotemia,

the urine osmolality exceeds iso-osmolar with plasma and creatinine clearance, is slightly diminished (Jones and Weil, 1971). Exceeding urine osmolality is an indication of extra-renal oliguria, since it can only be presented in the kidneys with adequate function. In contrast, an increase in U/P osmolality in our data may indicate the normal concentrating function of tubular as the glomerular filtration is diminished. Tubular reabsorption of water is also increased as shown in table 19, 23, 32 and 36. The onset of renal failure is more closly related to reduction of arterial blood pressure than to reduction of cardiac output (Jones and Weil, 1971). Futhermore, tubular damage is shown by inability of the kidney to excrete urea and creatinine, conserves and reabsorbs sodium, chloride and potassium, and extract potassium from the blood (Bull *et al.*, 1950). Recently, the direct nephrotoxicity effect of Russell's viper venom has been clear cut, since Russell's viper venom produces a dose dependent fall in creatinine clearance and fractional sodium excretion in the isolated perfused kidney rats (Ratcliffe and Pukrittayakamee, 1985). This obvious advocacy be similar to our results that decline in glomerular filtration may affect from prolonged renal vasoconstriction together with an increase in fractional sodium excretion which appear to be ATN (Bull *et al.*, 1950).

For renal pathological effects, our results are similar to the other investigators (Chugh *et al.*, 1975, 1977; Aung-Khin, 1978; Sarangi *et al.*, 1980). On the other hand, the Russell's viper venom 0.1 mg/kg bw. (iv) could not produce any renal lesions since being the minimal lethal dose (Tungthanathanich, 1983). Russell's viper venom and its metabolites maybe direct or indirect effect to the

renal cell (Sitprija and Boonpucknavig, 1979; Aung-Khin, 1978). Recently, I^{125} labelled Russell's viper venom injected at various doses were related to the radioactivity in the blood at different intervals (Thwin *et al.*, 1985). Therefore, at various doses of Russell's viper venom are able to produce the different degree of renal lesion changes as shown here. Massive tubular necrosis in renal medulla associated with frequent foci of ATN in cortex is prominently suggested that temporary lack of tubular blood supply to the renal tubules as well as focal tubular necrosis scattering in renal parenchyma. Hemorrhage in the glomeruli and interstitium maybe due to the hemorrhage resulting from consumptive coagulopathy as described by Aung-Khin (1978), however, no fibrin thrombi are seen so that vasculotoxic effect is possibly. Hemorrhagic necrosis of cortical region in the large dose of Russell's viper venom infusion group may possibly results from a profound shock, intravascular coagulation as well as hemolysis according to features of necrosis. Another discernible factor that is most likely to be involved including hemoglobinuria. Recently, an increase in plasma hemoglobin levels associated with hemoglobinuria have been found in the victims (Chugh *et al.*, 1984). Plasma free hemoglobin levels of greater than 200 mg/dl have been reported to be particularly toxic to the renal tubular epithelial cells (Yeh *et al.*, 1963). In our results, there are some invasion of segmented neutrophils in the tubule and interstitium. Bywaters and Stead in 1944 believed that the finding of pigment casts in the lumina of tubule produced the tubular obstruction and that is a major hemoglobinuric ARF. Additionally, tubular degeneration and necrosis are slightly more prominent in the direct venom infused kidney. By different doses of Russell's

viper venom, we found that in the small dose (0.05 mg/kg bw.) of Russell's viper venom produced the focal tubular necrosis while the focal cortical hemorrhagic necrosis was additionally in the large dose of venom (0.1 mg/kg bw.)

Our results have been suggested that renal hemodynamics and histopathological changes of oliguric renal failure are affected from two components, firstly, one is a profound shock that be primarily responsible for decrease in renal functions, and secondly, the rest maybe due to a direct nephrotoxicity together with shock, intravascular hemolysis and coagulation.