Chapter II

Background Information

Snakebite is a serious public health problem and a common medical emergency in tropical and subtropical countries and also in certain other parts of the world (Chugh et al., 1975; Jeyarajah, 1984; Schmidt et al., 1976; Shastry et al., 1977). The mortality from snakebite around the world has been estimated from 30,000 to 40,000 persons, annually. High mortality figures have been reported from India, Burma, Ceylon, Thailand, Malaysia and Brazil. There were 15,000 deaths occour in the indian subcontinent (Chugh et al., 1975), 0.9 per 100,000 population in Thailand (Trisnananda, 1979) and 1,000 cases in Burma annually (Thein-Than et al., 1985). The fatal cases from poisonous snakebite considerably trigger attention.

About 2700 species of snake in the world have been classified. Most of them possess non-poisonous snakes and the remains more than 300 species are poisonous. In Thailand, there are 122 species of snake and devided according to their poison into serious poisonous 10, mild poisonous 23, poisonous sea snake 24 and the remains are non-poisonous snakes. The poisonous snakes are classified into 3 families as follow :

Elapidae : The elapid snakes are land snake. They have relatively short fixed fangs, round bodies and head and have neurotoxic poison, they include cobras, kraits and coral snake.

Hydropidae : There are many species of sea snakes. Characteristic by flat and very short fixed fangs, prominent flattened

tailed and have myotoxic venom.

Viperidae : Snakes in Viperidae family have hematotoxic and cytotoxic venom. They are subdivided into crotaline and viperine viper. Snakes which belong to Crotaline subfamily possess the most sensitive and specialized thermal receptors in the cephalic region between eye and nostril. These receptors detect infrared radiation of relatively long wave length (Barrett et al., 1970). The receptor of crotaline viper is called the facial pit and sometimes pit viper is frequently substituted. By these receptors these reptiles were able to detect external thermal stimuli by their prey which helps them to hunt in the nights and also make them remarkably sentitive to changes in their thermal environment (Gopalakrishnakone, 1984). The pit viper include Malayan pit viper and green pit viper. The viperine viper are pitless. Both subfamily have similar long erectile fangs, triangular heads, distinct neck and usually short fat bodies. The most common and widely distributed spicies of viperine viper is the Vipera Russellii or Russell's viper or Tic-palonga or Kasari Hebi and or Daboia. It is found throughout the plains of India, Burma, Ceylon, Sumatra, Java and Thailand. The Russell's viper is generally of quiet and peaceful habits and prowls about at night in search of prey which consists of mice, rats, frogs, etc. It attacks man in self-defence and only when provoked. When ready to attack it produce a loud hissing sound, as air leak from the puncture wheel, which can be heard from a distance of 20-25 feet (Chopra and Chowhan, 1934). The maximum growth is about 5 feet long, Russell's viper is ovoviviparous and may have between 20-60 young at a time (Stidworthy, 1969).

Russell's Viper Venom.

The venom is yellowish liquid, its relative viscosity varies from 1.5 - 2.5 folds as compare to water (Devi, 1968). This venom is accumulated in the lumina of salivary glands and expelled during the bite. Most of the viper venoms are stored extracellularly in the glands. The amount of ejected venom is approximately 10 % of the storage venom (Bdolah, 1979), or 72 mg in a single strike, depending on age, size and emotion of the snake (Chatter Jec, 1965). Dried viperine venoms can be refigerated for 5 years without appreciable destruction of toxicity. The dried venom is not completely soluble in distilled water, 79.5 %, the solubility in physiological saline is much higher than that of distilled water, 93 % (Bucherl, 1968). In preliminary studies on Indian snake venom, the Russell's viper venom were found to contain the elements of C, H, N, S and O (Ganguly and Malkna, 1935). Iwanaga and Suzuki (1979) recently found that the organic component of Russell's viper venom are lipid, carbohydrate, amino acid, riboflavine, nucleosides, nucleotides and organic phosphate compounds. Additionally, metal inorganic component were also found as following : Na^{+} , K^{+} , Zn^{++} , Ca^{++} , Mg^{++} , Fe^{++} , Co^{++} and Mn^{++} (Gnasset et al., 1956; Devi, 1968). A number of enzymes which contribute to the patho-physiologic actions of the Russell's viper venom have been detected. They are include phospholipase A_2 , L-amino acid oxidase, phosphodiesterase, 5' neucleotidase, phosphomonoesterase, deoxyribonuclease, ribonuclease, adenosine triphosphatase, hyaluronidase, NAD - nucleosidase, arylamidase, peptidase, endopeptidase, arginine ester hydrolase, kininogenase, proteinase activators, proteinase inhibitors, thrombinlike enzyme,

factor X activator, factor IX activator, factor V activator, prothrombin activator and nerve growth factor (Iwanaga and Suzuki, 1979; Morris et al., 1978; Kisiel, 1979; Lindquist et al., 1978; Pearce et al., 1972; Takahashi et al., 1974). It is generally agreed that the enzymes in snake venoms act in the following ways : (a) local capillary damage and tissue necrosis by proteinases, phospholipases, arginine ester hydrolases and hyaluronidase (Slotta, 1955; Meaume, 1966; Suzuki and Iwanaga, 1970); (b) diverse coagulant and anticoagulant actions by various proteinases and phospholipase A (Meaume, 1966); (c) induce acute hypotension and pain due to release of vasoactive peptides such as histamine, 5-hydroxytryptamine, kinins, slow reacting substance (SRS), prostaglandins, (PGS) prostacyclin, (PGI_2) thromboxane A_2 (TXA_2) and leukotrienes by kinin releasing enzyme and phospholipases. (Suzuki and Iwanaga, 1970; Huang, 1984; Huang and Lee, 1984). In addition to the enzymes, many other proteins have been isolated from Russell's viper venom. The protein components of the venom can be classified into three groups : (a) proteins with toxic properties such as membrane toxins which act on various membranes increasing their permeability, (b) proteins with enzymic activities, and (c) proteins with not known biological activities (Devi, 1968). It is know that the toxicity of Russell's viper venom has long been suggested to be due to some enzymes rather than polypeptide toxins.

Absorption of the Venom

Barnes and Trueta (1941) studied the absorption of elapid venoms in rabbits after subcutaneous injection. By blocking the

lymphatic circulation of the region into which the venom was delayed as judged from the delay of death. The action of cobra venom, however, was not hindered. They speculated that the absorption of tiger snake venom is mainly from the lymphatic system presumably because of its high molecular weight whereas cobra venom can be absorbed through the capillaries due to smaller molecular weight of its neurotoxin and the other components quite through lymphatically. Russell's viper venom, however, the information is still lacking. Since the molecular sizes of Russell's viper venom is nearly to the other species, some author speculated that the absorption is analogy.

Distribution of the Venom

Than and Co-worker (1985) studied the distribution of ^{125}I labelled Russell's viper venom in mice following intramuscular administration. The radio-tagged venom was found in all organs at all time intervals. The stomach, kidney, liver and lung were found to reach peak levels by 4 hr, 8 hr in the spleen, heart, testes and brain, and 16 hr. in the thyroid and intestine. In the kidney, however, there was a sharp rise in venom levels at 24 hr until 48 hr after the first initial decline. The biphasic pattern of distribution displayed by the kidney may reflect late renal involvement in the animals envenomated with Russell's viper venom. Amount of the venom in muscle tissue at the site of injection was declined with times interval, they suggesting that early removal of venom from the site of bite would effectively reduce the amount of venom available for further spreading into the body compartments and the pattern of distribution is dose-related. Furthermore, they reported that the

amount of venom accumulated in the brain was lowest and insignificant at all time intervals, according to the previous reported in crotalid venoms (Gennaro and Ramsey, 1959; Huang et al., 1972; Wingert et al., 1980). It seems likely that the Russell's viper venom, like crotalid venoms, may have little action on the central nervous system. It is apparent that the venom polypeptides pass the blood - brain barrier with difficulties, but it is not true to say that none of them can penetrated the blood - brain barrier (Chang, 1979).

Fate and Excretion of the Venom

Shu et al (1968) and Huang et al (1972) have shown that radiolabelled venoms were excreted in the urine and feces of animals. Recently, Than et al (1985) have been reported that the greater fraction of administered venom was taken up by the stomach, kidney, intestine and liver. They proposed that these tissue involve in the elimination of venom during postenvenomation. The major route of elimination of labelled venom was through urinary excretion. The fecal excretion of venom was relatively less important. It appears that the administered venom may be picked up by the liver and excreted in the intestine via bile secretion.

Symptomatology

It is generally described the manifestations of snake bite as local versus systemic ones. This classification is certainly justified, mainly for didactic reasons, but it does not mean that the chronologic order of appearance of manifestations is necessarily.

Local symptoms : The victim apparents pain and swelling at the

site of the bite 2 - 3 min after the bite, simultaneously with bleeding from fang marks. Typical fang marks are rarely encountered. Usually, a single skin puncture or scratch is present. Soon swelling of the bitten extremity sets in. The swollen part is tender and painful to palpation. The swelling subsequently increases and spreads centripetally, reacting tremendous dimensions during the following days and usually clears after 1 - 2 weeks. A few days after, the reddening of the skin increases in width, ecchymosis and hemorrhagic blisters may appear caused by extravasation of red blood cell and plasma into the subcutaneous tissue. The contribution of the hyaluronidase and kininogenase toward the pain. swelling and extravasation of blood,reasons by hydrolyzed of hyaluronidic acid gel in the connective tissue by the enzyme hyaluronidase causes facilitated diffusion of the venom to the neighbouring tissue and circulation. Moreover, shifts to bradykinin of kininogen in the surrounding tissue by kininogenase and the releases of histamine from the mast cell cause increased vascular permeability and activated pain receptors at the bite site. The blisters are frequently ruptured and superficial necrosis usaully appears. If secondary infection is not involved, the wound usaully clear in 2 - 4 weeks. Tu et al (1969) reported that Thailand Russell's viper (Russell's viper siamensis) venom caused myonecrosis and thrombus after intramuscular injection into mouse legs.

Systemic symptoms : The victim soon becomes overwhelmed by weakness and restlessness 15 - 20 min after the bite. Repeatedly vomiting profusely perspiration, complains abdominal pains, echymosis, peticheal hemorrhage, conjunctiva hemorrhage, hematemesis,

epistaxis, hemoptysis, bleeding per gum hematuria and electro cardiogram (EKG) shown elevate ST segment and T wave were encountered. The sanguinolent diarrhea or watery and profuse like in cholera often reported. At this stage, the victim is usaully in severe periferal circulation failure. Hypotension is present and often the blood pressure cannot be measured. No pulse is palpable and heart rate is rapid. This marked hypotension may, however, pass over to hypovolemic shock caused by extravasation of blood into soft tissues and/or bleeding into some internal organs and intestines. The hemotological abnormalities in the victim are compatible by disseminated intravascular coagulation (DIC), prolonged coagulation tests such as clotting time, partial thromboplastin time (PTT), prothrombin time (PT), thrombin time (TT), thrombocytopenia. hypofibrinogenemia, increase fibrinolytic activity and depletion of factor X and V. These anomalous are a consequence of intravacular coagulation (Mersky, 1976). Schistocytes, anisocytosis and poikilocytosis are also manifested. A severe anemia is also developed a few days following the envenomation and/or bleeding into some internal organs and intestines. Occationally in certain cases agioneurotic edema of the lip, tongue and face may be observed (Mahasandana et al., 1980; Efrati, 1979). Several hours after the bite one can observe ascending lymphangitis and lymphadenitis on the affected extremity. There was high incidence of acute renal failure following Russell's viper bite (Chugh et al., 1975; Shastry et al.. 1977). Circulatory failure and acute renal failure was frequently caused of death.

Hemodynamic Effects

Chopra and Chowhon (1934) have been studied the action of Russell's viper venom on the circulatory system by intravenously administration a small dose of 0.05 - 0.1 mg/kg of the venom into a cat produce a slight initial rise in blood pressure followed by a gradual fall to 20 - 30 mmHg. With larger dose of 0.2 - 0.5 mg/kg, the fall was more pronounced and the blood pressure remained permanently at low level. At dose of 0.5 mg/kg. Russell's viper venom produced an immediate and irreversible decline in arterial blood pressure and the dog sometimes died suddenly from convulsion and heart failure (Lee, 1948; Vick et al., 1967). Similary. from Tungthanathanich et al (1986) in conscious dogs. the animal recieved 0.45 - 0.5 mg/kg of venom intravenously all died within 48 hr and the animal sometimes died suddenly from convulsion and heart failure. The sudden death of rabbits produced by Russell's viper venom was proved to be due to intravascular coagulation (Lee, 1944).

Recent investigation attempted to elucidate the mechanism responsible for impairment of renal function during envenomation. A minimal lethal dose of 0.1 mg/kg of Russell's viper venom was given intravenously in anesthetized mongrel dogs (Tungthanathanich et al., 1986). Two phases of changes in cardiovascular system were observed.During initial postinjection period. mean arterial blood pressure, total peripheral resistance and renal vascular resistance were increased. The decreased in blood pressure and heart rate were sustain for 30 min and returned to normal value within 2 hr after venom administration. The pattern of the changes in the first phase are similar to the study in rats (Chaiyabutr et al.. 1985). The

decrease in blood pressure and heart rate during the first phase has been found to be due to the vasovagal effect (Chopra and Chowhan, 1934; Lee and Lee, 1979), which could be prevented by vagotomy (Lee and Lee, 1979). Vick and Co-worker (1967) believed that the hypotension was attributed to vasodilation of capillaries in the hepato-splanchnic area and this was prevented by evisceration. However, this conclusion may not apply to the recent results, since in dogs either splenectomy (Tongvongchai, 1984) or intravascular volume expansion with dextran solution (Chaiyasest, 1986), a marked decrease in blood pressure and cardiac output were still apparent after envenomation. Tongvongchai (1984) pointed that the spleen was not the major contributor to the total blood volume shifts caused by the effect of the venom. Somehow, other organs and/or venous vascular bed may play an important role (Shoukas et al., 1981).The hypotensive action of Russell's viper venom remains doubtful as to whether affect the heart directly to decrease cardiac output and blood pressure or whether the release or formation of pharmacologically active autacoids. Vipera russellii venom contains several isoenzymes of phospholipase A_2 (Salach et al., 1971). Most of phospholipase A_2 of the venom had hypotensive actions in rats given 0.1 mg/kg intravenously (Huang and Lee, 1984). Huang (1984) suggested that phospholipase A_2 fraction in the venom released autacoids such as histamine, 5-hydroxytryptamine (5-HT), kinins, slow reacting substance (SRS) and prostaglandins (PGS) which associated with a number of pathologic processes involving blood pressure, pulmonary function, vascular permeability and visceral smooth muscle tone. Moreover, releases of thromboxane A_2 (TXA_2), prostacycline (PGI_2), histamine

and leukotrienes from perfused guinea-pig lungs which might cause vasodilation in the periphery, combine with pulmonary vasoconstriction that restricted blood return to the heart, leading to a decrease in cardiac output and induce a greatr hypotensive effects have been reported by Huang (1984).

The rise in blood pressure following the transient decrease after envenomation was due to released of cathecholamine as a compensatory mechanism. According to Chaiyabutr and his colleagues (1984) that increase in systemic vascular resistance and packed cell volume after Russell's viper venom injection. Failure to compensate would lead to death (Chaiyabur et al., 1984).

The second phase, 8 - 48 hr. after envenomation, the cardiac output increased the total peripheral resistance and mean arterial blood pressure were restored to normal level (Tungthanathanich et al., 1986). They proposed that the changes in this period are not only induced by catecholamines release but by the other hormones. Renin angiotensin system is belived to be the main mechanism since increased in the renin activity, reasonably by the converting enzyme inhibitor (MK-422, enalapril maleate) could be decreased the blood pressure without compensation in either pre- or post - envenomated administration (Chaiyabutr et al., 1985).

Russell's Viper Venom and Hematological Abnormalities

It has been known for many years that the venom of Russell's viper has powerful coagulant properties (Lamb and Hanna, 1903; Barnett and Macfarlane, 1934). Moreover, it has been suggested by several

investigators, that the venom can be used as a local hemostatic for hemophilies diseases and a substitute for tissue thromboplastin in the determination of the prothrombin time (Barnett and Macfarlane, 1934; Page et al., 1942; Witts and Hobson, 1940). Rosenfeld et al (1986) pointed out the similarity between venom and tissue extracts injected in vivo. From these observations thromboplastin like activity was ascribed to venom. Also, Arthus, (1919) and Houssay and Sordelli, (1914-1920) consisdered the venom as thromboplastin because it requires calcium.

Biggs and Co-workers (1953) and Rapaport and associates (1954) reported that the venom show an activity equivalent to factors V, VII, VIII and IX, which are activated in the early phase of the clotting mechanism. Furthermore, Esnouf and William (1962) can be purified factor X and the coagulant factor in Russell's viper venom.

In present, generally, know that the acceleration of blood coagulation by Russell's viper venom is mainly by active proteins which might affect different steps in the blood coagulating cascade (Teng et al., 1984). The active proteins were known as activating enzymes, usaully, protease as follows : prothrombin activating enzyme (Teng et al., 1984), factor V activating enzyme (Kisiel, 1979), factor IX activating enzyme (Lindquist et al., 1978; Byrne and Castellino, 1978), factor X activating enzyme (Di Scipio et al., 1977; Moris et al., 1978). The thrombin like enzymes, α -, β - fibrinogenase, platelet aggregation inducers and factor VII, VIII activators are also included. The prothrombin inhibitors, platelet aggregation inhibitors and anticoagulant protein have been purified from Russell's viper venom (Teng et al., 1984). Interference in blood coagulation by the

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venom would aggravate bleeding, and acceleration of blood coagulation by the venom might cause disseminated intravascular coagulation or bleeding after consumption of the coaculating factors (Teng et al., 1984). The consequence of intravascular coagulation included thrombocytopenia (Chugh et al., 1975), prolonged coagulation tests (Mahasandana et al., 1980), rised in serum fibrinogen degradating products (Rehman et al., 1984) and depletion of factor II, V, VIII, X (Mahasandana et al., 1980; Sitprija et al., 1980, 1982). These are the main causes of death in bitting by the Russell's viper snake (Teng et al., 1984).

Massive intravascular hemolysis was found in the case of Russell's viper bite (Mahasandana et al., 1980). Because of snake venoms contain direct and inderect lytic factors. Direct lytic factor (DLF), itself can hemolyze the red cell. Indirect lytic factor lyses the red cell very slowly, however, its lytic action can be greatly intensified by the addition of phosphatidylcholine. The indirect lytic factor is identified as phospholipase A_2 (Tu, 1977). When phospholipase A_2 and direct lytic factor are combined, the mixture becomes strongly hemolytic. Venom of Russell's viper contain phospholipase A_2 but not direct lytic factor (Tu, 1977). Thus Russell's viper venom exerts indirect hemolytic effect. It has been shown by Condrea (1979) that the total mass of human red cell membrane consists of 49.2 % protein, 43.6 % lipid and 7.5 % carbohydrate. Phosphatidylcholine (Lecithin), sphingomyelin and phosphatidyl ethanolamine are the major red cell phospholipids which good substrates for phospholipase. Another major cause of hemolysis in envenomation is disseminated intravascular coagulation and this was

protected by preventing clotting with heparin (Brain et al., 1957). Infusion of thrombin could be produced hemolysis in the further experiment. Later, Mckay et al (1970) suggested that intravascular hemolysis caused by snake venom might be attributed to at least three mechanism : (a) the action of phospholipase resulting in the detachment of globular red fragment, (b) disseminated intravacular coagulation and (a) increased fragility of spherocytes in their ordinary passage through the vascular system.

Intravascular hemolysis can be evaluated by the degree of hemoglobinuria (Condrea, 1979). Chugh et al (1975) demonstrated that the patients with Russell's viper bites whom developed intravascular hemolysis and disseminated intravascular coagulation lead to the development of acute renal failure.

Nephrotoxicity of Russell's Viper Venom.

The effect of Russell's viper venom on the kidney are most complex. Renal involvement is usually in the form of acute renal failure that is an important cause of death in patients who have been bitten by Russell's viper snake (Sitprija et al., 1974). A broad spectrum of renal lesions including tubular necrosis (Sitprija et al., 1974; Sitprija and Boonpucknavig, 1977), cortical necrosis (Chugh et al., 1975), glomerulonnephritis (Sitprija and Boonpucknavig, 1980) and acute interstitial nephritis (Sitprija et al., 1982) have been reported. Russell's viper venom contains hematotoxin and cytotoxin which affects the whole bodily functions. Many investigators have been believed that acute renal failure cause by Russell's viper venom is secondary to intravascular hemolysis and intravascular coagulation

(Chugh et al., 1975; Sitprija and Boonpucknavig, 1979). Hypotension due to the cardiovascular effect of the venom can cause the sudden decrease in renal blood flow which may induce ischemic renal failure (Reid et al., 1963; Schrier and Conger, 1980). However acute renal failure have been noted in some cases of Russell's viper bite without hypotension (Sitprija and Boonpucknavig, 1979) and no correlation between the severity of renal failure and coagulopathy (Shastry et al., 1977). From these reasons, the investigators propose that Russell's viper venom may play a directly role in cytotoxic effect on renal tubular cell or vascula wall and conduct to the development of acute renal failure.

A number of studies attempted to elucidate the mechanism of Russell's viper venom induce acute renal failure. They are divided into histopathological, immunological and physiological study.

Histapothological changes :

In order to better understand the nephrotoxic action of Russell's viper venom, therefore, it seem necessary to scrutinize in detail the histopothologic changes, pathogenesis and manifestation of the involved kidney.

Glomerular lesions : The predominant lesions consist of mild proliferation of mesangial cells with an increase in the amount of basement membrane like matrix. Inflammatory exudate, hemorrhage in glomerular tuffs and glomerular edema are also found. Occationally, there are polymorphonuclear cells in the glomerular capillaries (Sitprija and Boonpucknavig, 1979). In addition, marked vascular and parenchymal congestion was seen. There are also showed fibrin thrombi

in the vessels (Chugh et al., 1975). Sitprija and Boonpucknavig (1980) reported extracapillary proliferative glomerulonephritis in theglomeruli. Neither immunoglobulins nor complement (C_3) were detected but immunofluorescence showed fibrin deposition in the glomeruli. They suggested that the glomerular changes presumably due to the direct effect of venom, the mesangial proliferation being a non-specific pathological reaction. Since Russell's viper venom is vasculotoxic it could cause rupture of the glomerular basement membrane with fibrin deposition and secondary epithelial porliferation.

By electron microscopy, occational narrowing of the glomerular capillary lumen was observed. This to be due to mesangial hyperplasia with an increase in the amount of basement membrane like matrix (Sitprija and Boonpucknavig, 1979). Chugh et al (1978) showed the cytoplasm of Bowman's capule epithelium appeared swollen and contained numerous organells. A microvillous border (Brush border) was pressent on the parietal epithelial surface in some areas. They described here corresponds to " tubularization ", a well-known characteristic in acute tubular necrosis, Moreover, the urinary space contained epithelial cell cytoplasmic fragments, extruded erythrocytes and fibrin were also pressent. Glomerular capillaries showed areas of irregular glomerular basement membrane thickening and wrinkling, total endothelial swelling and occasional degenerating endothelial cells. The lumen contained granular meterial, clusters of platelets and occasional degenerating neutrophilic leukocytes. The presence of these damaged glomeruli demostrate that the glomerular injury may sometimes be irreversible and in severe case result in cortical necrosis (Date and Shastry, 1982).

Sitprija and Boonpucknavig (1979) have been proposed three theories to explain the glomerular pathogenesis in Russell's viper bite. (1) direct irritation by snake venoms, (2) fibrin deposition, that conceivable to be a subsequence of intravascular coagulation and led to glomerular damage and (3) immunologic reaction by the venom serve as an antigen.

Vascular lesions : The most obvious alteration was necortizing arteritis of the interlobular arteries. The lesion was found segmentally. Thrombophlebitis of the arcuate vein and its tributaries were also presented. The vasculotoxic properties of the deep vein and artery by Russell's viper venom has been suggested to be a direct toxic action on the kidney by the venom which not found in non-specific toxic vasculitis (Sitprija and Boonpucknavig, 1977, 1979). Electron microscopy showed swollen of endothelial and cytoplasmic protrusion into the lumen, the cytoplasm contained numerous vacuoles and a dilated smooth endoplasmic retuculumn. Medullary blood vessels were sevrely affected with markedly swollen focally necrotic endothelial cells almost completely obliterating the lumen. Occational platelet clusters were also presented (Date and Shastry, 1982).

Tubulointerstitial lesions : Acute tubular necrosis was well documented in the cases of Russell's viper bite (Chugh et al., 1975; Date and Shastry, 1982; Sitprija and Bonpucknavig, 1977, 1979, 1980; Sitprija et al., 1974, 1982; Shastry et al., 1977; Jeyarajah, 1984). The proximal, distal and collecting tubules were dilated and lined by flattened epithelial cell with deeply stain nuclei. Eosinophilic granular casts were seen in some tubules (Sitprija et al., 1974).

Chugh et al (1975) and Date and Shastry (1982) showed the uniform debasement membrane and denudation of tubular epithelium which led tubular leakage of the tubular fluids into interstitium that well known in the term " tubulorrhexis ". This characteristic usually found in tubularnecrosis probably caused by interruption of tubular blood supply at the glomerular level (Chugh et al., 1975; Date and Shastry, 1982; Kreisberg, 1983). In electron microscopic study showed numerous dense intracytoplasmic bodies, presumably representing degenerating organelles. Occasional tubular cells showed extrusion of their cytoplasmic contents into the tubular lumen and there were granular material and cytoplasmic debris contained in tubular lumen (Date and Shastry, 1982).

Interstitial changes were observed only in cases with renal failure. A variety of in flammatory cells were also presented, including numerous eosinophils, mast cells, plasma cells, lymphocytes and some macrophages and basophils. Mononuclear cells are more dense in the interstitial tissue of corticomedullary junction and always located around the necrotic collecting tubules. Interstitial lesions were infact secondary to tubular necrosis (Chugh et al., 1975; Sitprija and Boonpucknavig, 1979). Recently, Sitprija et al., 1982 reported that, there was intense and diffuse interstitial cellular infiltration while the mild infiltration seen in tubular necrosis. Eosinophils, immlunoglobulin and complement (C_3) were not detectable in the interstitium unlike drug induced interstitial nephritis. The changes cannot be explained by immune complex mechanism, immediate type of hypersinsitivity or delayed hypersensitivity reaction. They suggested that the interstitial nephritis could represent a reaction

to the Russell's viper venom that attributed to a direct nephrotoxicity.

Cortical necrosis : Bilateral massive cortical necrosis was observed by Chugh et al(1975) in patients with Russell's viper bite. The characteristic ghostlike appearance of glomeruli and tubules in the necrotic areas was a common feature. This lesion is usually associated with severe disseminated intravascular coagulation and tubular necrosis (Sitprija and Boonpucknavig, 1979).

Immunological Reaction :

It is well established that glomerulonephritis can be produced by immunologic mechanism. In Russell's viper bite, the antigen-antibody complex may trigger the immunologic reaction leading to the development of glomerular lesion similar to those observed in infections diseases (Sitprija and Boonpucknavig, 1979). Sitprija and Boonpucknavig (1974) reported necrotic of arteriolar wall and glomeruli show deposition of β - 1 C globulin without immunoglobulins, according to the decreased in serum β - 1 C globulin. They suggested that the nonimmunologic activation of the complement system through the alternate pathway. The immunologic mechanism of the venom was short duration, therefore, glomerular lesions were mild and transient. Severe glomerulonephritis from the Russell's viper venom could be predominantly due to direct glomerular irritation by specific component of the venom (Sitprija and Boonpucknavig, 1979).

Physiological Studies :

In order to attempt to explain the cause of impairment of the renal function during Russell's viper envenomation, three groups of

experimental approach were classified.

Studied on renal hemodynamics : It is conceivable that changes in renal functions are associated with changes on cardiovascular system. Circulatory failure may lead to acute renal failure which caused by filtration failure due to reduce renal circulation. Many investigators have been studied the effect of Russell's viper venom on renal hemmdynamic and cardiovascular system (Chaiyabutr et al., 1984; Tungthanathanich, et al., 1986; Tongvongchai, 1984). They showed that the venom caused an obviously decrease in general circulation and renal hemodynamic fallowing the initiate of envenomation. The blood pressure gradually increased and aproaced the control level with in 2 hours. However, renal blood flow, glomerular filtration rate and renal fraction (% cardiac output) were decreased throughout the period of 2 - 24 hr after given a minimal lethal dose of 0.1 mg/kg of Russell's viper venom (Tungthanathanich et al., 1986). They proposed to be due to local vasoconstrictor released in the kidney which associated with an increased in renal vascular resistance. Direct evidence for the involvement of renin-angiotensin system in response to renal vasoconstriction after envenomation has been proposed by Chaiyabutr et al (1985) in pretreated envenomize rats with intrarenal angiotensin II blockage (MK-422, enalapril maleate). Resulted in increased in urine flow, glomerular filtration rate and renal blood flow were demonstrated, when compared with the nonpretreated rats.

According to current concepts, renal circulation regulated by two hormones system. Vasoconstriction, mediated by norephinphrine and/or the renin-angiotensin system; while prostaglandin compounds and the kallikrein-kinin system act as vasodilators. Interestingly,

Tongvongchai (1984) demonstrated the pretreated animals with indomethacin, an inhibitor of prostaglandin synthesis, alleviated the hemodynamic effect of Russell's viper venom in both intact and spleenectomized dogs. A posible endogenous mechanism for releasing the hormone induce vasocostriction after envenomation may be due to the lack of dilatory prostaglandins (e.g. PGE_2) and/or overproduction of thromboxane A_2 (TXA_2), a powerful renal vasoconstriction (Gerber et al., 1978). Whether an activation of renin-angiotensin system are mediated indirectly through the action of catecholamines (Vander, 1965) or prostaglandins (Werning et al., 1971) remain to be defined.

Studied on renal functions : It is well-established that impairment of renal function can be produced by Russell's viper venom. Renal involvement in mains usually in the form of acute renal failure. The mechanisms operate in consert in the acute renal failure remains unclear. In general, felt that all of the reported in cases of acute renal failure from Russell's viper bite were defined from the clinical studied (Sitprija and Boonpucknavig, 1974, 1979, 1980; Sitprija et al., 1982; Jeyarajha, 1984; Shastry et al., 1977; Date and Shastry, 1982 Chugh et al., 1978). No evidence of acute renal failure have been reported in experimental model. Tungthanathanich et al (1986) and Tongvongchai (1984) showed that the tubular function decreased at 2 hr after envenomation and returned to normal condition at 24 hr. However, the renal vascular resistance was still at high level and the renal perfusion decreased untill the end of the experiment. They suggested that acute renal failure may be caused by the long-time ischemia. Since the experiments were performed in a short period, however, acute renal failure provided more time to open. Recently,

Retcliffe and Pukrittayakamee (1985) demonstrated in the isolated perfused rat kidney, showed some light that Russell's viper venom produced a dose dependent fell in inulin clearance and rose in fractional excretion of sodium significantly similar to that seen in acute tubular necrosis. They concluded that caused by the direct nephrotoxicity of the venom.

Studied the effect on cellular level : Very few data are available on the renal micropuncture and cellular level study in experimental animals given Russell's viper venom. Among the deficit, Chaiyabutr et al (1985) studied on electrophysiology by measurement of peritubular transmembrane potential. They found the venom induced depolarization of the proximal tubular cell in similar pattern to the 2-4-dinitrophenol (DPN), inhibitor of aerobic phosphorylation in renal tubular cell which might reflect the arrest of an electrogenic sodium pump. The venom interact directly with specific sites that control the transport of ion across the tubular cell was suggested. Further studies are needed to elucidate whether a change in cell membrane potential during venom perfusion could result in an elevated intracellular specific ions, since particularly elevated intracellular calcium has been shown to destroy normal cell functions (Sarkadi et al., 1982). On the other hand, intracellular ions might be increased from the venom stimulated the ATPase enzyme system in tubular cell as found in liver mitochondria (Sattyev et al., 1974). The exact mechanism or combination of these mechanism by which the venom depolarized the tubular cell membrane still remain to be clarified.

Factors Influence Renal Handling of Inorganic Phosphorus

It has been recognized inorganic phosphorus was reabsorbted in the proximal tubular brush border membrane by the sodium-phosphate cotransport system (Denis et a., 1979). Inhibition of sodium dependent inorganic phosphate transport could be induced by parathyroid hormone, which affectd through adenylate cyclase of the basolateral membrane of the renal proximal tubular cell. Then intracellular c-AMP increased, and enhanced phosphorylation of brush border protein by membrane bound c-AMP dependent protein kinase accompanied by change in membrane permeability (Hammerman and Hruska, 1982). Partially, the intracellular c-AMP is leakage into tubular lumen, which could be estimated in terminal urine (Butten and Jard, 1972). The infusion of dibutyryl c-AMP either systemically or directly into the renal artery inhibited proximal tubular sodium dependent inorganic phosphate reabsorption, resembling the effects of parathyroid hormone qauntitatively and qualitatively (Agus et al., 1971). Jastack and Co-worker (1968) has been documented that acute renal insufficiency could lead to secondary hyperparathyroidism. Moreover, under acidotic condition inorganic phosphorus, provides the major source of non ammonium urinary buffer, was many folds increased for buffered renal hydrogen ion excretion (titratable acid) (Hulter, 1984). Strickly linear relationship was found between the urinary inorganic phosphorus excretion and urinary titratable acid excretion (Gyory et al., 1968).