CHAPTER II

BACKGROUND INFORMATION

Snake bite poisoning is becoming an increasingly significant health hazard in the tropical and subtropical regions of the world, although the problem is not limited to them alone. The world mortality from snake bites has been estimated at 30,000 - 40,000 annually (Chugh *et al.*, 1975) , out of which in Thailand alone, the annual mortality rate due to snake bites is about 0.9 per 100,000 population (Trisnananda, 1979). All of the common poisonous snakes viz. vipers, cobras and kraits, the Russell's viper is the commonest offending snake in Thailand and the fatal cases from snake bite poisoning considerably attract attention.

Medically important snakes have fangs at the front of their mouths which enable them to inject venom efficiently. These are the "poisonous" snakes and there are three families as follows:

1. The Elapidae Family

The elapid snakes are land snakes. They have relatively short fixed fangs; they include cobras, mambas, kraits, coral-snakes, and all the Australian poisonous snakes.

2. The Hydrophidae Family

The hydrophidae consists of many species of sea snakes. They

have very short fixed fangs and characteristic flat, prominent fattened tails; they are common in Asian coastal waters.

3. The Viperidae Family

The viper snakes have long, erectile fangs, triangular heads, are usually short fat bodies. Vipers are subdivided into crotaline or pit vipers, having a thermosensitive pit between eye and nose, and viperine vipers, without pits. The pit detects warm-blooded prey in the dark (Khan, 1983). *Vipera russelli* is the same as *Indian doboia russelli* (Chopra and Chowhan, 1934). It is named *Vipera russelli* in Burma, *Vipera russelli formosensis Maki* in Taiwan and *Vipera russelli siamensis* in Thailand (Lee, 1948; Mahasandana *et al.*, 1980).

Russell's viper is very common in all parts of Thailand. It is generally of quiet and peaceful habits and prowls about at right in search of prey which consists generally of mice, rats, frogs, lizards, ants, eggs etc. It attacks man in selfdefence and only when provoked. When ready to attack it produces a loud hissing sound which can be heard from a distance of 20 to 25 feet (Chopra and Chowhan, 1934). The snake venom is secreted from the salivary gland. This venom is yellow liquid, accumulated in the lumina of the glands and expelled during the bite. Most of the viper's venoms are stored extracellularly in the venom glands. A considerable amount of venoms are accumulated in the extended gland lumina (Sobol-Brown *et al.*, 1975). The removal of the venom from the gland lumina causes a renewal of venom production. The control mechanisms operating in synthesis and secretion in the venom glands are not well-understood. Sectioning of the main nerve supply to the venom glands does not

affect venom production, protein concentration or enzyme activities of the venom (Iwanaga and Zuzuki, 1979). It may thus be assumed that the control of venom production and secretion occurs in the glands themselves. It is evident that the amount of venom present in the lumina of the gland regulates the synthetic activity of the secretory epithelium and also its morphological structure (Kochva, 1960). The activation of the secretory epithelium in these glands induces an accelerated rate of protein synthesis, which is followed by secretion of venom into the gland lumen. Very little secretion is stored intracellularly and it seems that the secretion rate is correlated to the rate of venom production.

The venom spent in the bite seems to be derived from only a few tubules. Preliminary autoradiographic studies with glands after a bite indicate that only the epithelial cells of the tubule from which venom had been withdrawn are activated (Kochva *et al.*, 1975). Under natural conditions, viper usually has glands filled with venom, and during the bite only about 10 percent of the available venom is injected (Kochva, 1960). The maximum dose of a full grown Russell's viper can inject is about 150 mg to 250 mg of the venom at a single bite while the average dose given at a single bite is about 72 mg (Chopra and Chowhan, 1934).

According to epidemiological report, the rural hazard is due to snake bite. Most bites are found at ankle or foot because the victim treads on or near the snake. The severity of poisoning is not related to the time of the bite, breeding habits of the snakes, or the age of the victim (Reid, 1968).

Venom Components and Activities

A prelimlnary investigation on the nature of the chemical elements in the doboia venom (Russell's venom) led to the identification of the following: C, H, N, S and O; with trace of inorganic substances and colouring matters (Ganguly and Malkana, 1936). Crude venom is fractionated into seven fractions by DEAE cellulose (Panichakul, 1967). He found that there were only five active toxic principle in five fractions. The activity of phosphodiesterase, phosphomonoesterase, adenosine triphosphatase were in fraction I, and phospholipase A was in fraction V. The toxicity of crude venom was more than each fraction. Potisook (1972) has studied the toxicity and enzyme activities of the fractionated parts of the crude venom. Russell's viper venom was chromatographed on DEAE - cellulose column and fractionated into six fractions. Crude venom was found to be rich in amino acid esterase, phospholipase A, deoxyribonuclease, 5'-nucleotidase and exonuclease. It was found that all venom fractions were toxic and only fraction I, II and III were associated with phospholipase A, deoxyribonuclease, and exonuclease activities. He suggested that their lethality might be correlated to the actions of these enzymes. Recently, Iwanaga and Suzuki in 1979 have published and reported that the organic components of Russell's viper venom were lipid, carbohydrate, amino acid, nucleotides nucleosides and organic phosphate compounds. Additionally, there are at least seventeen enzymes which most of them are hydrolase, of these enzymes are phospholipase A_2, phosphodiesterase, α-amino acid oxidase, 5'-nucleotidase, phosphomonoesterase, deoxyribonuclease, ribonuclease,

adenosine triphosphatase, hyaluronidase, NAD-nucleosidase, arylamidase, endopeptidase, arginine ester hydrolase, kininogenase and factor X activating enzyme. Several workers have tried to correlate the toxicity of a particular snake venom with its enzyme activities. It is generally agreed that the enzymes in snake venom act in the following ways:

1. effect on local capillary damage and tissue necrosis by proteinase, phospholipase, arginine ester hydrolase and hyaluronidase (Slotta, 1955; Suzuki and Iwanaga, 1970).

2. cause diverse coagulant and anticoagulant actions by various proteinase and phospholipase A_2 (Mcaume, 1966; Boquet, 1964).

3. induce acute hypotension and pain. These effects seem due to release of vasoactive peptides by kinin releasing enzyme (Suzuki and Iwanaga, 1970) and phospholipase (Rothschild and Rothschild, 1979).

These enzymes are widely varying biological and pharmacological properties. Indirect effects of snake venoms are due to the release or formation of pharmacologically highly active autacoids such as histamine, 5-hydroxytryptamine (serotonin), kinin, slow reacting substance (SRS), prostaglandins (PGs) (Iwanaga and Suzuki, 1979; Huang, 1984a). These secondary substances have been associated with various physiological process involving blood pressure, vascular permeability and other organ function changes. These are relatively few detailed reports. Most of the reports are surveying about the enzyme activities of a wide range of snake venom. The

physiological changes following phospholipase A and factor X activating enzyme activities of Russell's viper venom are mostly reported.

Russell's viper venom is known to contain at least four enzymes which act on the hydrolysis of phosphate esters including endonuclease, phosphodiesterase, 5'-nucleotidase and phosphomonoesterase (Iwanaga and Suzuki, 1979). α-amino acid oxidase has been widely used in biochemical studies for identification of laboratory preparation of α-keto acid from α-amino acid. This enzyme is responsible for the yellow colour in snake venom because it contains FAD as the prosthetic group (Iwanaga and Suzuki, 1979). The enzymes that act on glycosyl compounds are hyaluronidase and NAD-nucleosidase (Iwanaga and Suzuki, 1979). Hyaluronidase is defined as an enzyme that catalyzes the clevage of internal glycosidic bonds of certain acid mucopolysaccharides of animal connective tissue, e.g. sodium hyaluronic acid and sodium chondroitin sulfate A and C. It is widely distributed in mammalian testes, many invasive bacteria and animal venoms (Meyer *et al.*, 1960; Iwanaga and Suzuki, 1979). It is referred to as "spreading factor" because hydrolysis of hyaluronic acid facilitates toxin diffusion into the tissue of a victim (Duran-Reynals, 1936). NAD-nucleosidase catalyzes the hydrolysis of the nicotinamide N-ribosidic linkage of NAD and has been isolated from various mammalian tissue (Iwanaga and Suzuki, 1979). Russell's viper venom contains several proteases including endopeptidases, proteinase with a limited specificity such as kininogenase, arginine ester hydrolase, factor X activating enzyme. Venom endopeptidase catalyzes the hydrolysis of peptide bonds of a wide variety of natural and synthetic substrates including casein, hemoglobin,

collagen, fibrinogen, bradykinin etc. (Iwanaga and Suzuki, 1979). Kininogenase cleavages plasma kininogen to form bradykinin, a vasoactive nanopeptide. In preliminary study, Rocha *et al.* (1949) have reported that the venoms of certain snakes, as well as the enzyme trypsin, acted on plasma globulin to produce a substrate, probably a polypeptide, that also diminished blood pressure and caused a slowly contraction of gut. Bradykinin is defined as Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, a amino acid sequence (Sander and Huggins, 1972). The plasma bradykinin forming system contributed blood clotting and fibrinolysis through the Hageman factor (HF) - dependent process (Esnouf, 1982).

Russell's viper venom also contains several isoenzymes of phospholipase A_2 (Salach *et al.*, 1971). Recently, the venom was separated by means of Sephadex G-75 column chromatography into five fractions (Huang and Lee, 1984). Phospholipase A_2 activity was concentrated in fraction II and III. Pharmacological properties of phospholipase A_2 in snake venom was shown to have a variety of actions, for example hypotension (Ho and Lee, 1981), neurotoxicity (Lee and Ho, 1982), local capillary damage and tissue necrosis (Suzuki and Iwanaga, 1970) and anticoagulant action (Boffa *et al.*, 1982). Huang (1984b) studied the ability of phospholipase A_2 fractions to release slow reacting substance (SRS) in guinea pig lungs which perfused with Kreb's solution and the components of slow reacting substance were identified. Phospholipase A_2 induced an increase in the lung perfusion pressure accompanied by a release of prostaglandins-like and leukotriene-like substance and also histamine. He suggested that phospholipase A_2 fractions from

Russell's viper venom released arachidonic acid from membrane phospholipids which was transferred to thromboxane A_2 (TxA_2) and prostacyclin (PGI_2) by cyclooxygenase or to leukotrienes by lipooxygenase. Slow reacting substance is identified as a mixture of thromboxane A_2 (TxA_2), prostacyclin (PGI_2) and leukotrienes, all of these autacoids releasing from lung increase vascular permeability and may be affected in pulmonary edema formation (Huang, 1984b). It is well-known that histamine and prostacyclin have direct vasodilatory properties (Gryglewski, 1979; Abboud, 1983). Recently, Leukotriene was shown to have a prolonged hypotensive effect in guinea-pig and monkey. (Drazen *et al.*, 1980; Piper *et al.*, 1981; Smedegard *et al.*, 1982). Therefore, part of the hypotensive action of phospholipase A_2 fractions may be due to the actions of histamine, prostacyclin and leukotriene released. It is generally that phospholipase A_2 catalyzes the hydrolysis of the fatty acid esterified at the second position of phosphoglycerides, giving rise to unsaturated fatty acid and 1-acylglycerophosphatides. Phosphatidylcholine (α-lecithin) is the most common substrate for venom phospholipase A_2 (Iwanaga and Suzuki, 1979). The hydrolysis of this substrate can take place in free substrate form in serum (Tu *et al.*, 1968). Hemolysis by viper venom is due to the action of phospholipase A_2 indirectly (Condrea, 1976; 1979). This venom enzyme has been designated as phospholipase A, phosphotidase A, lecithinase A or hemolysin because of its ability to hemolyse red blood cell directly (Iwanaga and Suzuki, 1979).

Most reports on the study of toxicity of the Russell's viper venom were powerful coagulant properties. Esnouf and William in 1962 purified both factor X and the coagulant factor in the venom,

and showed that the factor X molecule was split enzymatically to form a product of lower molecular weight which had both coagulant and esterase activity. Kisiel (1976) purposed factor X activator from Russell's viper venom was a highly specific protease composed of one heavy chain and one light chain, and these chains were held together by a disulfide bond (s). The factor X activating enzyme contains 13% carbohydrate including 6% hexose, 1.7% N-acetylneuraminic acid and 5.3% galactosamine. Most of the carbohydrates are found to be present in the heavy chain, although some are also observed in both forms of the light chain (Kisiel, 1976).

Among the wide variety of biologic substance which present in snake venom, nerve growth factor (NGF) is one of the most curiousity. As it is presently understood, NGF is glycoprotein that regulates the growth and differentiation of neurons of sympathetic and sensory neural tissue (Angeletti, 1968; Banks *et al.*, 1968; Pearce *et al.*, 1972). The molecular weight of NGF is 37000 which determined by ultracentrifuge (Pearce *et al.*, 1972). Some details of Russell's viper NGF was pointed out by Iwanaga and Suzuki (1979).

Today, we know that Russell's viper venom is a complex mixture of components, many of which are known to be proteins possessing enzymes activities. The toxicity of Russell's viper venom has long been suggested to be due to some enzymes rather than polypeptide toxins.

Hemorrhagic and Necrotizing Effects

Hemorrhagic and necrosis are the most striking manifestations evoked by viperid venom. The local lesions are produced by snake venoms consist of edema, hemorrhage and necrosis (Ohsaka, 1979). Local swelling is the first sign that has been observed in patients (Reid, 1968; Mahasandana *et al.*, 1980). In human victim who is survived from snake bite, necrosis is seen at the site of the bite, with destruction of soft tissue (Reid, 1968). Hemorrhage and necrosis have been believed to be due, at least in part, to the action of proteolytic enzymes (Tu *et al.*, 1967). Experimental induction of myolysis (necrosis) with venom phosphodipase A (Sarkar and Devi, 1968) or lysolecithin (Phillips *et al.*, 1965) has prompted the suggestion that the necrotic effect of snake venoms is attributed to phospholipase A. Ownby *et al.*, (1976) have isolated a myotoxin from *Crotalus viridis* venom by gel filtration on Sephadex G-50, followed by chromatography on sephadex G-25. They found hemorrhage was not observed whereas injected muscle revealed a series of degeneration. Many proteinase such as trypsin, chymotrypsin in amount more than 50 ug induce necrosis on the back skin of the rabbit so that the proteolytic enzymes in snake venoms may also contributed to this effect (Takahashi and Ohsaka, 1970). Further elucidation of the nature of venom components that induce necrosis obviously depends on the advising of a reliable method for quantitative assay of necrotic activity.

The hemorrhage following viper bites produces both local and systemic bleeding in various organ such as heart, brain, kidney, intestine, lung (Efrati and Reif, 1953; Reid, 1968; Mahasandana *et al.*, 1980; Sarangi *et al.*, 1980). Hematemesis was the commonest symptom of the systemic bleeding (Reid, 1968; Mahasandana *et al.*, 1980; Sarangi *et al.*, 1980) induced prolonged coagulation tests such as clotting time, partial thromboplastin time, prothrombin time and thrombin time (Mahasandana *et al.*, 1980). Increment of fibrinolytic activity and depletion of factor X and V are also observed (Mahasandana *et al.*, 1980). Indian Russell's viper venom consists of a mixture of toxic proteins and enzymes which have hematotoxic and necrotizing properties (Chugh *et al.*, 1975). A few experimental performance are carried out by several investigators. The methods for determining hemorrhagic activity were skin reaction and lung surface reaction (Ohsaka, 1979). The hemorrhagic skin reaction method was defined as minimum hemorrhage dose (MHD) which at least amount of venom (ug dry weight) injected intradermally into rats and resulted in a hemorrhagic lesion of 10 mm cross-diameter on the inner side of the skin 24 hours later. Bonta *et al.* (1970) developed a method that allowed quantitive assessment of hemorrhage induced by application of snake venoms to the lung surface of opened-chest dog's preparation, and most evidences were generally evaluated by skin reaction. Chopra and Chowhan (1934) have shown the effect of doboia venom on blood coagulation. They found that the coagulation time was distinctly increased after administration of doboia venom in sublethal dose. It also showed that in each of the cases where the rabbit's ear was pricked, there was increased tendency to

hemorrhage and difficulty in stopping the oozing. At that time, hematoma was readily formed at the site of the prick. On post-mortem examination, extensive clotting was always formed in the veins and pulmonary arteries, some petechial hemorrhages in lung, endocardium, intestinal submucosa and kidney (Lee, 1948). Viper venom damages the blood vessels and especially to the arterioles and capillaries (Efrati and Reif, 1953). Ganguly, 1936 has suggested that proteoses fractions contain the hemorrhagic toxin of the venom and more intensed reaction in the presence of albumin. Tu *et al.* (1967) studied six venoms of Thailand origin and concluded that hemorrhagic activity had good correlation with proteolytic activity. Although the ratio of LD_{50} to the minimum hemorrhagic dose (MHD) of Russell's viper venom is very small but the hemorrhagic activity is also distributed in all venoms of both crotalidae and viperidae (Ohsaka *et al.*, 1966). Ohsaka *et al.* (1960) reported the absence of proteolytic activity from a hemorrhagic toxin in *Trimeresurus flavoviridis*. The contradictory statements were suggested by Huang and Perez, (1980). They have shown the hemorrhagic and proteolytic activity of snake venoms were not closely related since the neutralization with the sera was different. Ohsaka et al. (1973) succeeded in isolating three hemorrhagic principles, HR1, HR2a and HR2b, from the venom of *Trimeresurus flavoviridis*, a crotalid. The purified HR1 contains some proteolytic activity on casein while HR2a and HR2b are freed of this activity. Pharmacological studies on the hemorrhagic principle recently isolated from snake venoms have contributed a great deal to the understanding of the physiological mechanisms involved in hemorrhage. Cinematographic studies revealed the specific mode of action of the venom

hemorrhagic principles on the microcirculatory system. Ohsaka in 1976 reported that the early effects of the crude venom and purified hemorrhagic principle were characterized by severe vasoconstriction, followed by vasodilatation, of larger vessels especially of arterioles and subsequent hemorrhage in the capillary bed. The erythrocytes spurted one by one through pin-point holes form in true capillaries whereas the true capillaries with hemorrhagic lesions increase in number of time. The formation of white thrombus (platelet aggregate) is seldom observed at the site of venom-induced hemorrhage. No sign of increased vascular permeability for albumin prior to the onset of erythrocyte spurting. Electron microscopic observations recently demonstrated that the erythrocytes, changing their shapes in common with amoeba, spurted through opened junctions of endothelial cell lining, the adjacent basement membrane being disrupted to permit eventual extravasation of the erythrocytes. *In vitro* study, they also showed the ability of the venom principles disrupted the isolated basement membrane. The basement membrane was considered to constitute a solid barrier against erythrocyte spurting. Ohsaka *et al.* (1973) purposed and put forward a hypothesis that hemorrhage was evoked by exogenous hemorrhagic principle of enzymic nature. It was also interesting to test the venom hemorrhagic principles for their inhibitory effects on the hemostatic mechanism constituting the secondary cause of hemorrhage, namely disturbance of blood coagulation and inhibition of platelet aggregation. They reported that the purified hemorrhagic principles inhibited ADP-induced platelet aggregation while no appreciable effect on blood coagulation was observed. Further study and

investigation on general physiological mechanisms involve in hemorrhage will be proved and discussed.

Hemolytic Effect

Snake venoms have been shown to contain factors which are able to attack and disorganize cellular and subcellular membranes. Although snake venoms are heterogenous in composition and produce multiple simultaneous effect, venoms of snakes belonging to the elapidae family is predominantly neurotoxic whereas viperidae and crotalidae venoms cause mainly hemorrhage and coagulation (Condrea, 1979). Red cell lysis which may develop following snake bite does not play a prominent role in overall venom lethality, its contribution being slight as compound with the venom effects on the vessel walls, coagulation, heart, nervous system etc. Hemolysis in circulating blood is more probable to occur following bites by elapidae than by viperidae or crotalidae (Condrea, 1979).

Hemolysis is the expression of red cell membrane destructed by the action of these venom factors which act either directly or through a complex multistep process. The hemolytic property of the Russell's viper venom was tested by Iswariah and David (1932) in various concentration on the erythrocyte of dog and man. In concentration of 1 in 1000 or more, the red blood cell hemolysed in 5 to 15 minutes. In higher dilutions, no hemolysis was noticed even in 2 to 4 hours. Chopra and Chowhan (1934) emphasized that the hemolying property of the venom could be activated by the presence of lecithin or substances containing lecithin such as bile, hot milk, cephalin. Cholesterin acts in an antagonistic manner to lecithin, and its

presence in the blood prevents hemolysis. They attributed that the erythrocytes were hemolysed by the venom only if they were not properly washed. The hemolysis was due to the susceptibility of the red blood cells which were insufficiently washed. While viperidae venom could hemolyse the red blood cells indirectly with the presence of lecithin, only a number of venoms belonging to the elapidae family could hemolyse the washed red cells directly without any addition. The explaining direct hemolysis is through direct lytic factor, a polypeptide devoid of enzymatic activity (Condrea, 1979). Today, the basic polypeptide devoid of enzymatic activity is designated as cardiotoxin, skeletal muscle-depolarizing factor, cobramine A and B, cytotoxin, toxin γ, peak 12 β, direct lytic factor (DLF), membrane-active polypeptide (Lee, 1972; Condrea, 1974; 1979). Phospholipase A_2 and direct lytic factor are the main active component of venom in hemolysis (Condrea, 1974). Direct lytic factor is, by itself, only weakly hemolytic but act synergistically with phospholipase A (Lee, 1972; Condrea, 1974).

According to a modern concept, the membrane components are organized in a fluid mosaic of lipid and protein, the structure is stabilized by lipid-lipid and protein-protein interactions and by Ca^{++} mediated bonds (Singer and Nicolson, 1972). Damaging structural membrane is lost permeability property and transport capacity resulted by entry of water and the red cell swelling, stretching of the membrane and eventually hemolysis, respectively (Rosenberg, 1976; Condrea, 1979). Condrea *et al.* (1962) have reported that the serum protein promoted hemolysis. Indirect hemolysis test is performed on washed red cells and added plasma, hemolysis can be induced whereas

the amount of plasma is sufficiently high (Condrea, 1976). Two-step processes of hydrolysis are occured, the first is hydrolysis of exogenous lecithin or phosphotidylcholine to lysolecithin and fatty acid and the second, the red blood cells are lysed by these lecithin split products (Condrea, 1979). The total mass of the human red cell membrane consists of 49.2% protein, 43.6% lipid and 7.5% carbohydrate. Phosphotidylcholine (Lecithin), sphingomyelin and phosphotidyl ethanolamine, major red cell phospholipids, are good substrates for phospholipase (Condrea, 1979). Another major cause of hemolysis in envenomization is disseminated intravascular coagulation (Chugh *et al.*, 1975; Harris *et al.*, 1976; Kaplinsky *et al.*, 1980; Mahasandana *et al.*, 1980). Although it is clear that the phospholipase component of snake venom is responsible for some hemolysis *in vivo*, it is also shown that this is not the role mechanism of hemolysis. By using *Echis colorratus* venom in dogs, Klibansky *et al.* (1966) showed that injection with phospholipase A containing boiled venom which was devoid of procoagulant activity caused hydrolysis of plasma lecithin, attachment of lysolecithin to the circulating red cell, crenation and spherocytosis, but little or no intravascular hemolysis. However, the procoagulant containing venom fraction which was devoid of phospholipase A produced disseminated intravascular coagulation and intravascular hemolysis without changing in plasma phospholipids or red cell morphology by light microscopy. It has been shown by Brain *et al.* (1967) that disseminated intravascular coagulation caused intravascular hemolysis and this was protected by preventing clotting with heparin. In further experiment, they produced hemolysis by infusion of thrombin. McKay

et al. (1969) have observed that the continuous infusion of high doses of epinephrine produced intravascular coagulation and hemolysis. Later, they attributed both to be mediated indirectly by the activity of the microcirculation induced by epinephrine because both were prevented by adrenergic blockade. They considerably suggested that intravascular hemolysis caused by snake venom might be attributed to at least three mechanisms: (1) the action of phospholipase resulting in the detachment of globular red fragments, (2) disseminated intravascular coagulation and (3) increased mechanical fragility of spherocytes in their ordinary passage through the vascular system (McKay *et al.*, 1970).

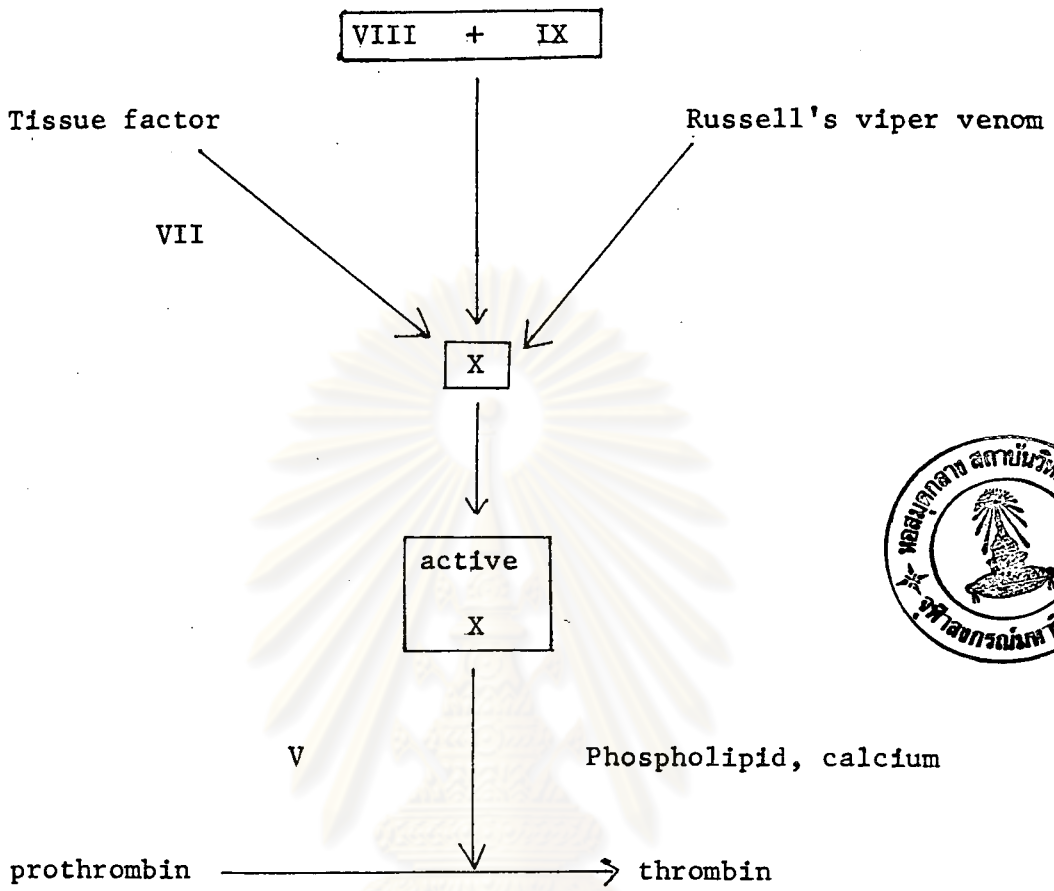
Intravascular hemolysis can be evaluated by the degree of hemoglobinuria (Condrea, 1979). According to clinical reports of Russell's viper victims, frank hemoglobinuria has been recorded (Reid, 1968; Peiris *et al.*, 1969). The three out of eight patients with acute renal failure following Russell's viper bites demonstrate intravascular and disseminated intravascular coagulation which lead to the development of acute renal failure (Chugh *et al.*, 1975).

Intravascular Coagulant Effect

Vipera russelli venom has been known for many years as a powerful coagulant property. It is the outstanding example of the venom that generates Autoprothrombin C (factor Xa) from its precursor. In preliminary study, Lamb and Hanna in 1903 have shown that injection of venom into animal intravenously caused massive thrombosis. When the venom was tested on oxalated or citrated blood *in vitro* it had little coagulant activity. Using heparin and other anticoagulants

prove that the sudden death produced by *Vipera russelli* venom is due to intravascular coagulation (Lee, 1944), and the same conclusion is also reached by Ahuja in 1946. Lee *et al.* (1955) demonstrated that the *Vipera daboia formosan* venom had a potent coagulant action *in vivo* as well as *in vitro* and that its action resembled that of thromboplastin but not that of thrombin. It is very interesting that the venom reacts with factor X in serum or with factor X preparation to form an active product which is a potent prothrombin activator in the presence of phospholipid, factor V and calcium (Macfarlane, 1964). The factor X has been suggested to act as a substrate of the coagulant factor in the venom (Macfarlane, 1964). Esnouf and William (1962) purified both factor X and the coagulant factor in the venom and showed that factor X molecule was split by this coagulant factor to form a product of low molecular weight. Using fractionated preparation, Macfarlane in 1963 has shown an incubating mixture of factors VIII, IX, X and calcium developed coagulant activity resembling that produced by a mixture of venom and factor X. It also appeared that factor VIII and IX underwent some reaction to produce an "activator" of factor X. Macfarlane (1964) suggested that factor X was activated by the intrinsic system of blood clotting from the coagulant factor in venom. According to a brief historic background which was written by Lee *et al.* (1955), the quotations were followed: Bigg cited the work of Treven and Macfarlane, who showed that venom was mostly effective together with lecithin. Later, Leathews and Mellanby observed that lecithin augmented the thromboplastic activity of the venom, but that of brain extract to a lesser degree. Macfarlane, Treven and Attword removed lipoidal components of horse plasma

by prolonged centrifugation at high speed. When subsequently recalcified, the plasma could no longer be coagulated by addition of daboia venom unless lecithin was also added. Quick also showed that the prothrombin time of human plasma normally which was twelve seconds, may be reduced to three seconds if lecithin and venom were used concurrently. He also remarked tissue extracts were not affected by lecithin, and that the venom, in contrast to tissue extracts. Eagle stated that *Vipera daboia* venom did not clot oxalated plasma, hence it did not activate fibrinogen. Today, we reasonably know factor X is an important link (both the extrinsic and intrinsic blood coagulation system) in the mechanism of normal prothrombin activation. Factor X deficiency prolongs the one-stage prothrombin time and produces abnormal results in the prothrombin consumption and thromboplastin generation test. The Russell's viper venom became commercially available under the name 'Stypven'. For the purpose of using 'Stypven' as a substitute for thromboplastin in the one-stage prothrombin time is the control of hemophilic bleeding (Macfarlane, 1967). Thus, Macfarlane (1964) purposed a hypothetical representation of the ways in which factor X was activated by factor X activating enzyme in venom to form activated factor X which could convert prothrombin to thrombin.



The venom was attributed to be a cytotoxic agent enhancing the process of platelet disruption and thereby liberating what was considered to be thromboplastin (Ganguly, 1936). In controversy, Lee *et al.* (1955) demonstrated that the venom did not require purified preparation of platlet factor III for activation of purified prothrombin. Davey (1965) reported that Russell's viper venom caused platlet aggregation only at the lower concentrations tested (5-20 ug per ml), but releasing nucleotide could be observed with all concentrations. Aung-Khin (1977) has studied the effect of Russell's viper venom on blood coagulation, platelets and fibrinolysis *in vivo* and *in vitro* and pointed it out as a strong coagulant.

At the time of manifestation of bleeding from consumption coagulopathy, the most striking hemostatic abnormalities were the fall of fibrinogen level, reduction in platelet count, delayed ADP aggregation of platelets, increased fibrinolytic activity and presence of fibrin degradation products. These findings showed that Russell's viper venom interfered with blood coagulation and was the cause of platelet function abnormalities and led to activate the fibrinolytic enzyme system. No thrombin-like activity is found in this venom (Sakuragawa *et al.*, 1979). Coagulation-fibrinolysis is strongly activated and it sequently brings on platelet aggregation occuring in the body (Sakuragawa *et al.*, 1979). The presence of fibrin thrombi has been observed both in experimental animals (Taylor and Mallick, 1935; Aung-Khin, 1978) and in clinical cases of Russell's viper bite (Chugh *et al.*, 1975), consequently support the occurence of disseminated intravascular coagulation (Chugh *et al.*, 1979). Reid *et al.* (1963) have firstly reported a defibrination syndrome following envenomation by Malayan pit viper. McKay and Margaretten (1967) explained that intravascular coagulation produced a characteristic sequence of changes in components of the hemostatic mechanism. Initially, there was a decrease in platelets, circulating fibrinogen, prothrombin complex, factors V, VII, VIII and X. This depletion was due to the fact that these substances were used up in the process of coagulation and from the activation of plasminogen with the formation of active fibrinolysin which further diminished the concentration of fibrinogen and certain other factors by enzymatic degradation. Intravascular clotting and fibrinolysin activation usually occurred simultaneously (Mckay and Margaretten, 1967; Deykin, 1970).

Coagulation disorders might be occurred in three patterns: (1) hypofibrinogenemia or afibrinogenemia without thrombocytopenia, (2) disseminated intravascular coagulation (DIC) without thrombocytopenia and (3) Hypofibrinogenemia with secondary fibrinolysis (Kaplinsky, 1980). In some clinical cases, mild hypofibrinogenemia and thrombocytopenia have been reported (Tallon *et al.*, 1981). Deykin (1970) has described an activated factor XII, by activating the kallikrein system, resulted in the elaboratation of bradykinin. The venom could release some autacoids such as kinin, histamin, 5-HT, SRS, prostaglandins, which were vasodilatory effect (Huang, 1984a). Russell's viper venom has also hematotoxic, vasculotoxic and cytotoxic properties (Reid, 1968; Chugh *et al.*, 1975). Thus, hypotensive status certainly occurred. These complex factors may operate to trigger the blood coagulation and fibrinolytic system contributing to the severity of intravascular coagulation (McKay and Margaretten, 1967; Deykin, 1970).

Cardiovascular Effect

Snake venoms have a variety of dangerous effects on almost every organ systems. In most cases of snake venom poisoning, circulatory disturbance is one of the most frequently encountered events. The effect of Russell's viper venom on the circulatory system was extensively studied by Chopra and Chowhan (1934). A small dose of Russell's viper venom (0.05 - 0.1 mg/kg) was injected intravenously into a cat produced a slight initial rise of blood pressure followed by a gradual fall amounting to 20 - 30 mm Hg. With larger doses (0.2 - 0.5 mg/kg), the fall was more pronounced and the blood

pressure remained permanently at a lower level. Rapid administration of large doses could produce a sudden fall in blood pressure and the animals sometimes died suddenly from convulsion and heart failure. Using the gradully increased dose of venom, the animals developed a sort of tolerance to it. They emphasized the symptoms of shock were due to the local dilatation of the capillaries of splanchnic area because there was enormous engorgement of the abdominal viscera and the collapse went hand in hand with hyperemia of the splanchnic area. If the mesenteric arteries were clamped, quite large doses of the venom would not have produced any marked effect in the blood pressure. This result is also reached (Lee, 1948; Vick *et al.*, 1967). The Russell's viper venom produces a pooling of the blood in the hepato-splanchnic bed of the dog (Vick *et al.*, 1967). Spleen is not the major contributor to the total blood volume shifts after envenomation (Tongvongchai, 1984). Concerning the volumes of visceral organs, Chopra and Chowhan (1934) have reported the volume of the intestine was markedly increased when the blood pressure decreased and the limb volume showed no markedly changed, whereas the kidneys and spleen volumes were decreased. In controversy, Iswariah and David (1932) reported a decrease in blood volume of splanchnic organ was in consequence of a fall in blood pressure. They suggested that the fall of the blood pressure was not due to active dilatation of the arterioles of the splanchnic region. Evisceration is able to prevent the initial hypotension and bradycardia (Vick *et al.*, 1967). The hypotensive action may not relate with reflex impulse and the nervous centers are not much affected, because the same results are obtained in decerebrated animals (Chopra and Chowhan, 1934; Lee, 1948). The paralytic action of the

venom on blood vessels is shown to be confined only to the capillaries in the perfusion experiment (Iswariah and David, 1932; Chopra and Chowhan, 1934). The veins and arteries are not dilated and showed a tendency to constrict. Capillaries paralysis following venom perfusion has been observed similar to that of histamine, since the venom is not able to give any fall in blood pressure after administration of large doses of histamine and vice versa (Iswariah and David, 1932). Histamine produces a profound fall in blood pressure by dilatation of the capillaries of the splanchnic area and not of the arteries or arterioles. The fall of blood pressure is due to dilatation of the capillaries although the arterioles are actually contracted (Chopra and Chowhan, 1934; Lee, 1948). Drugs like ether and chloroform which depress the capillaries will potentiate the action of the venom. Adrenalin and pituitrin which tone up the capillaries tend to rerive the blood pressure after envenomation (Chopra and Chowhan, 1934). The capillaries paralysis may be responsible for excessive loss of fluid from the vascular system (Iswariah and David, 1932; Chopra and Chowhan, 1934). Death is preceded by spasmodic and irregular respiration, convulsion and asphyxia which these indicate the involvement of the vagal center owing to difficient blood supply (Chopra and Chowhan, 1934). The circulatory action of *Vipera russelli formosensis* venom, a subspecie of Russell's viper, was studied in rabbits by Lee (1948). This venom had a coagulant effect and produced intravascular clotting upon intravenous administration. Post mortem examination, some petechial hemorrhages were found in the lung, the endocardium, the psoas muscles and intestinal mucosa. No clots were found in the

heart or in larger blood vessels. A small dose of venom (0.01 mg/kg) was injected into rabbits intravenously and produced a slight fall in the blood pressure. The respiration has been slightly increased while the blood pressure was fallen off. The recovery of both, blood pressure and respiration, took place after ten minutes. With a sublethal dose (0.05 - 0.1 mg/kg), the blood pressure was gradually dropped and accompanied with an increasing heart rate. Subsequent injection of the same dose did not produce significant changes in either blood pressure or heart rate. Nevertheless, with the dose of 0.5 mg/kg, the blood pressure dropped rapidly to the zero line within a few minutes and no recovery was observed. Injection of adrenalin, isotonic saline or artificial respiration failed to restore the blood pressure. Section of both vagi and atropinization did not alter in any way of the venom action. Lee concluded that the venom caused a marked fall in blood pressure and a transient stimulation of respiration. He also believed the fall in blood pressure seemed due to peripheral action. On the other hand, in the animals pretreated with heparin (25 mg/kg), no sudden death is observed even though a larger dose (5 mg/kg) of the venom was given (Lee, 1944). The arterial blood pressure drops to lower level and after that, the animals are finally dead from circulatory failure (Lee, 1944). Lee (1948) has studied the hemodynamics effects of heat treatmented venom (at 80°c; for 30 min) destroyed the coagulant and most other enzymes. A large doses of heat venom (5-10 mg/kg) produced no sudden, as in the heparin pretreated animal, it produced an immediate and irreversible fall in the arterial blood pressure and also the animals died of circulatory failure within several hours. Regardless of whether a small

or large dose was administered, the volume of the small intestine was markedly increased. The liver volume also increased slightly whereas the kidney volume has shown falling off and the limb volume raised transiently following its decrease. None of elimination of the brain circulation, cutting of the carotid sinus and depressor nerves altered the hypotensive effects of the heat venom. He suggested that the death from Russell's viper venom was due to the at least following two components: One was coagulant enzyme and might be responsible for the acute sudden death due to intravascular clotting. The other was the thermostable vasculotoxic and produced a sustained hypotension led to delay circulatory failure (Lee, 1944). The hypotensive action of Russell's viper venom (0.05-1.0 mg/kg) was not due to central in origin and heat venom (80°c, for 30 min) did not appreciably affect the abrupt hypotensive activity. In the isolated heart of frogs and rabbits, the venom induced a stimulant action in small dose but an inhibitory action in larger dose. After that, the coronary outflow of the rabbit's heart showed a prompt increase following a fall in larger dose (Lee, 1948). Chopra and Chowhan (1934) showed the venom had no marked direct effect either on the myocardium or the nervous apparatus of the heart. The supportive evidence was also reported by Iswariah and David (1932). Vick *et al.* (1967) also reported that a lethal dose (0.5 mg/kg, iv) of Russell's viper venom produced an immediate and irreversible fall in arterial blood pressure whereas pulse pressure narrowed and heart rate has been decreased. Respiration was not affected during the initial post injection period, but after approximately 10 minutes, respiratory movements ceased abruptly and profound bradycardia was noted.

A rather slow progressive decline in arterial blood pressure occured over 15-30 minutes period. Vagotomy prevented only bradycardia but not arterial blood pressure. Heart rate was increased significantly after vagotomy was done. They attributed Russell's viper venom produced a pooling of blood in the hepato-splanchnic bed of the dog. Apparently, these authors did not consider the possibility of intravascular coagulation. Indirect effects of snake venoms were due to the release or formation of pharmacologically highly active autacoids which may contribute to the hypotensive action. Rosenberg (1976) has discussed the effects of phospholipase A_2 as the following categories: (1) Cell structure and permeability, including effects on various cellular membranes. (2) Effects on metabolic systems including mitochondrial enzymes. (3) Release of physiologically active compounds including acetylcholine, adrenalin, histamine and prostaglandin precursors from their tissue storage sites. (4) Effects of neurotransmitter receptors including the acetylcholine and GABA receptor. (5) Use as enzymatic probes to study phospholipid function in axons, synapses and the central nervous system. Some autacoids such as kinin was released by kininogenase in the venom (Oshima *et al.*, 1969), while histamine is also released by the venom (Fearn *et al.*, 1964), in common with prostacyclin and leukotrienes which are released by phospholipase A_2 (Rosenberg, 1976, Huang, 1984). All of these autacoids are able to fall blood pressure off (Armstrong *et al.*, 1978; Drazen *et al.*, 1980). Sket and Gubensek (1976) have demonstrated the hypotensive action from phospholipase A_2 fractions and crude venom of *vipera ammodytes*, using indomethacin as an inhibitor of prostaglandins synthesis. Both of the crude venom and

phospholipase fractions produced a fall in systemic arterial pressure. No effect on a fall in blood pressure while indomethacin (50 mg/kg) was given 15 min prior to the injection of the venom (0.5 mg/kg) or the phospholipase fraction (0.5 mg/kg). In view of the fact that indomethacin (50 mg/kg) did not abolish hypotension after administration of phospholipase fraction might indicate the existence of at least two different pharmacological effects of phospholipase A. One that could be blocked by indomethacin and the other which was resisted to its action. In a previous study, Huang and Lee (1984) documented phospholipase A_2 enzyme from Russell's viper venom was noted as being thermostable (70-80°c, 30 min, pH6.8) and having hypotensive action. This enzyme released thromboxane A_2, prostacyclin and leukotrienes from the isolated perfused guinea pig lung (Huang, 1984b). *In vivo* study, prostaglandins are widely known to participate in the regulation of vascular reactivity and blood pressure. They may act directly an vascular smooth muscle (Hatano *et al.*, 1980), by amplifying the effects of the kallikrein-kinin system (McGiff and Quilly, 1980), by modulating the action and/or release of noradrenalin from vasoconstrictor nerves (Hedquist, 1979) or by opposing the vasoconstrictor and anti-natriuretic actions of the renin-angiotensin-aldosterone system (McGiff *et al.*, 1970 a; Henrich, 1981). The kidney and blood vessels are major sites of prostaglandin synthesis (Nasjletti and Malik, 1982) and several prostaglandins are capable of affecting vascular and renal mechanisms involved in blood pressure control (Moncada and Vane, 1978). Prostaglandins also stimulate renin secretion and cause increase in blood pressure (Gerber *et al.*, 1978; Freeman *et al.*, 1984). On the other hand, angiotensin stimulates

the release of prostaglandin E_2 and prostacyclin that are capable of counteracting the vasoconstrictor action of this hormone (McGiff *et al*, 1970b; Shebuski and Aiken, 1980). The renin-angiotensin system might play an important role in blood pressure and water electrolyte balance. On the other hand, a naturally occuring renin inhibiting system was also described by several authors (Ostrovsky *et al*, 1967; Smeby *et al*., 1967). A phospholipid extracted from dogs and human inhibited renin *in vitro*. This phospholipid was considered to be a "pre-inhibitor", since renin inhibition was dependent upon its conversion to a lysophospholipid by endogenous phospholipid A. Therefore, phospholipase A_2 of snake venom might activate the renin pre-inhibitor. Recent work by Huang (1984b) showed the effects of phospholipase A_2 from Russell's viper venom on blood pressure, plasma prostacyclin level and renin activity in normotensive and hypertensive rats. He found that the hypotensive response to phospholipase A_2 was markedly reduced after administrating indomethacin (30 mg/kg, iv). There was the same result that reported in *Vipera ammodytes* venom (Sket and Gubensek, 1976). Phospholipase fractions (0.1 mg/kg) induced an increase in plasma prostacyclin and thromboxane A_2 levels and there was a positive linear correlation between the phospholipase A_2 -induced hypotensive effect and the ratio of increase 6-Keto-PGF_1 to thromboxane B_2 (TxB_2) in normotensive rats. In renal hypertensive rats, prostacyclin level was more increased than in normotensive rats. Plasma renin activity was reduced by phospholipase A_2 fractions in renal hypertensive rats, but not in normotensive rats. He emphasized the hypotensive effect of phospholipase A_2 fractions in normotensive rats may be partly due to the increase in plasma prostacyclin and

thromboxane A_2 (TxA_2) levels. In addition to the larger increase in plasma prostacyclin level, the reduction in plasma renin activity may also contribute to the greater hypotensive affect of phospholipase A_2 fractions in renal hypertensive rats.

Previously, the effects of Russell's viper venom on cardiovascular and renal hemodynamics system were studied in spleenectomized dogs and the indomethacin-pretreated dogs (Tongvongchai, 1984). The results showed the animals without pretreated with indomethacin produced a marked reduction in mean arterial blood pressure and recovered to control level in a long period as compared to the indomethacin-pretreated group. It should be noted that heart rate after envenomation was variable responsed in all groups. Recent investigation tried to elucidate the cardiovascular effects. A minimal lethal dose of 0.1 mg/kg of Russell's viper venom was given intravenously in anesthetized mongrel dogs (Tungthanathanich 1983). Two phases of cardiovascular changes, the first was in within 2 hours and the second was 2-48 hours, were observed. During initial postinjection period, mean arterial blood pressure was abruptly diminished whereas total peripheral resistance increased. Heart rate was attenuated. A fall in mean arterial blood pressure, and heart rate decreases were sustained for 30 minutes and returned to the normal value within the first phase. Chopra and Chowhan (1934) have suggested that these were due to the vasovagal effect. Cardiac output, plasma volume and blood volume decreased during the first phase and none of significant differences has been shown. Phospholipase A_2 fractions of venom increased lung perfusion pressure which may restrict blood return to the heart and led to a decrease in cardiac

output and indirectly induced a greater hypotensive effects (Huang, 1984). Chaiyabutr *et al.* (1984) attributed a compensatory mechanism was due to catecholamine released and other hormones involvement during the second phase. The supportable documents were increase in both systemic vascular resistance and packed cell volume. When the converting enzyme inhibitor (MK-422 or enalapril maleate) was given, in either pre or post envenomation, the systemic blood pressure decreased without compensatory mechanism. The renin-angiotensin activity was attributed and being the one main mechanism in this period.

Renal Pathophysiological Effects

There is a broad spectrum of renal involvement in snake bite. All structure may be affected depending upon the type of poisonous snake. Among the large, active and complex organs of the body, the kidneys recieve the largest amount of blood per unit weight of tissue (Starling and Lavett, 1962), consequently, the kidneys are frequently affected by viper bite. Moreover, a few data have been studied on this organ. The clinical syndromes in relation to viper bite are variable while only minor reactions consist of local pain, swelling and local bleeding, severe reactions including hemorrhage, hypotension, shock, respiratory failure and death. Hematological changes predominate in viper envenomation (Sitprija and Boonpucknavig, 1979). Acute renal failure (ARF) is a common complication of Russell's viper bite (Sitprija and Boonpucknavig, 1977, 1979; Harris *et al.*, 1976; Chugh *et al.*, 1975; Shastry *et al.*, 1977; Mahasandana *et al.*, 1980; Matthai and Date, 1981; Jeyarajah, 1984). It was noted that ARF was clinically seen only in patients bitten by Russell's viper and

sea snake (Sitprija, 1979). Renal failure may appear due to indirect sequence of disseminated intravascular coagulation, shock or intravascular hemolysis (Chugh *et al.*, 1975; Shastry *et al.*, 1977; Aung-Khin, 1978; Sitprija and Boonpucknavig, 1979). However, direct vasculotoxic and nephrotoxic actions of the venom itself remain a possibility (Harris *et al.*, 1976; Sitprija *et al*; 1974, 1982; Sitprija and Boonpucknavig, 1979, 1980).

The effects of snake venom on the kidneys reveal a most complex pattern. Several toxic components of venoms act either directly or indirectly on renal cell (Sitprija and Boonpucknavig, 1979). Raab and Kaiser (1966) demonstrated that there were increases in alkaline phosphatase (AP) and leucine aminopeptidase (LAP) activities, brush border and basolateral membranes enzymes, after administering sublethal dose of *Agkistrodon piscivorus* venom subcutaneously in urine of rats. They suggested that these results were due to renal tubular cell damage. The pattern of urinary excretion of this enzymes may be differed after administration of different nephrotoxic agents, depending on their precise site of action (Koseki *et al.*, 1980). Amount of LAP increases toward the end of the proximal tubule whereas AP distributes in accordance with the distribution of luminal surface membranes alongs the nephron (Koseki *et al.*, 1980). Hadler and Brazil (1966) have studied renal histopathological picture of crotoxin intoxication and compared with the result of human being bitten by *Crotalus durissus* venom. They documented that the early renal histopathological lesions consist of capillary congestion, thickness of the basement membrane, deposition of PAS positive material between the capilla-

ries loops and nuclear pycnosis of some glomerular cells. Damaged cells in distal convolutions were still less numerous. In late lesions, tubular damage predominated, the segment of the tubule mostly attained being the proximal one. They observed intravascular hemolysis and shock occured simultaneously besides renal lesions and these contributed to renal lesions changes. They believed that nephrotoxic substance might be responsible for tubular cell degeneration and necrosis. There were some evidences reported from two patients bitten by Russell's viper (Sitprija *et al.*, 1974). Both patients had bleeding symptoms presumably due to disseminated intravascular coagulation and mild intravascular hemolysis. Renal biopsy showed tubular necrosis and arteritis. The serum β-1C globulin was decreased whereas deposit in arterial lesion, the glomerular mesangium and arteriolar wall without immunoglobulins. They have suggested the non-immunologic activation of the complement system through the alternate pathway (Ruddy *et al.*, 1972) and the venom might be the activator. Venom changes have been documented by necrosis of the wall and luminal platlets thrombus occlusion. The deposition of β-1C globulin in the arteriolar wall differs from those of Efrati and Reif (1953) have describe that capillaries and arteriolars changes are characterized by endothelial swelling and tearing of the wall with hemorrhage. ARF is due to direct effect of arteritis which shows narrowing of the lumen and accelerates intravascular coagulation (Sitprija *et al.*, 1974; Sitprija and Boonpucknavig, 1979). Varagunam and Panabokke (1970) have attributed the vasculotoxic effect on the kidneys. They postulated that this effect was presumably due to absorption of the venom into blood stream and a very high concentration being achieved

by the kidneys because of their profuse blood supply. Anuria is commenced in the day that bitten and no vascular necrosis is found in the other organs (Reid, 1968). These are documents that support the vasculotoxic property of Russell's viper venom. Bilateral cortical necrosis or symmetrical cortical necrosis of the kidneys are also reported (Oram *et al.*, 1963; Varagunam and Panabokke, 1970; Shastry *et al.*, 1977). This term is used for indication of pathological entity of obscure etiology characterized by more or less complete and uniform necrosis of the cortex of both kidneys (Duff and Murrey, 1941). The necrosis may be ischemic in origin (Lauler and Schreiner, 1958). The most important single problem in renal failure is the development of cortical necrosis (Shastry *et al.*, 1977; Sitprija and Boonpucknavig, 1979; Sarangi *et al.*, 1980). Acute tubular necrosis always appears common renal lesion (Chugh *et al.*, 1975; Shastry *et al.*, 1977; Sitprija *et al.*, 1974; Sitprija and Boonpucknavig, 1977, 1979, Jeyarajah, 1984). Acute tubular necrosis is definited as anatomically the characteristic lesion which includes a necrosis and subsequent regeneration of the renal tubular epithelium, causes from poisonous toxin and prolonged renal ischemia (Bull *et al.*, 1950). Chugh *et al.* (1975) described that the striking histopathological features of direct nephrotoxicity in patients whom became total anuria with acute tubular necrosis. They have shown a uniform debasement and disapperance of tubular epithelium. Sarangi *et al.* (1980) documented the pattern of renal changes consisted of acute tubular necrosis, interstitial inflammation, edema and hemorrhage, glomerular swelling and aneurysmal dilatation of glomerular capillaries and cortical necrosis. They emphasized these renal lesions were due to

hemorrhage resulting from consumptive coagulopathy, vasculitis and direct nephrotoxicity. Scattered reports concerning the renal lesions include arteritis (Sitprija *et al.*, 1974 Sitprija, 1979), tubular necrosis, cortical necrosis, extracapillary proliferative glomerulonephritis (Sitprija and Boonpucknaving, 1980), acute interstitial nephritis (Sitprij *et al.*, 1982), glomerular mesangial proliferation and thrombophelbitis (Sitprija and Boonpucknavig, 1977). Acute interstitial nephritis and extracapillary proliferative glomerulonephritis are suggested in addition to tubular necrosis (Sitprija and Boonpucknavig, 1980; Sitprija *et al.*, 1982). The findings expand the spectrum of renal changes in Russell's viper bite including with changes in glomerular, vascular and tubulo-interstitium (Sitprija and Boonpucknavig, 1979). Multiple factors include direct tubulotoxicity, intravascular hemolysis, hypovolemia and disseminated intravascular coagulation are responsible for the development of renal changes (Sitprija and Boonpuckvanig, 1979). Since Russell's viper venom is vasculotoxic, it could cause rupture of glomerular basement membrane with fibrin deposition and secondary epithelial proliferation (Sitprija and Boonpucknavig, 1980). Seedat *et al.* (1974) postulated that the *Bitis arietans* (puff adder) venom produced the proliferative nephritis on a hypersensitivity basis. Although mild interstitial nephritis changes may occur in tubular necrosis around the degenerated tubules, interstitial nephritis is not common (Sitprija *et al*, 1982). Interestingly, there is intense, diffuse interstitial cellular infiltration unlike the mild infiltration in tubular necrosis and the cells are mostly mononuclear but no immunoglobulin or C3 are detectable in the interstitium (Sitprija and Boonpucknavig,

1980). According to the clinical symptoms, the course of renal failure was seldom prolonged comparing with tubular necrosis in the usual cases of Russell's viper bite and consistent with severe acute interstitial nephritis (Sitprija and Boonpucknavig, 1979, 1980). Glomerular mesangial lysis is produced by Habu venom (Brafield *et al.*, 1977) and rattlesnake venom (Schmidt *et al.*, 1976), believed to be the result of direct cytotoxicity. It is generally, snake venoms produce toxic nephropathy or toxic nephritis (Schreiner and Maher, 1965). Sitprija and Boonpucknavig have concluded interstitial nephritis could represent a reaction to the venom and was due to direct nephrotoxic effect. On the other hand, Aung-Khin (1978) has demonstrated general features of DIC, consumptive coagulopathy and paradoxic hemorrhage so were seen as a result of the strong coagulant effects of the venom. The silent features were intraglomerular deposition of fibrin, fibrin degradation products and coagulation. He described the obstruction of glomerular capillaries by coagulated material was the most likely cause of reduction of blood supply to the renal tubule. This tubular ischemia resulted in tubular necrosis and subsequent renal failure. He also noted that Russell's viper venom was a very potent coagulant and the mesangial cell (the mononuclear phagocytic system in the glomerular) could not remove the fibrin. Morever, the venom or its metabolites might have a direct toxic effect on the mesangial cell. Glomerular coagulation implicates in the pathogenesis of acute tubular necrosis (Clarkson *et al.*, 1970). Intravascular coagulation enhances intravascular hemolysis (Brain *et al.*, 1957) and intravascular hemolysis also causes intravascular coagulation (Deykin, 1970). These changes

are able to create a vicious cycle which enhance the cause of renal failure (Sitprija and Boonpucknavig, 1979). In severe hemolysis, tubular obstruction by the casts might cause renal failure (Steinhausen *et al.*, 1982). There is no correlation between the severity of renal failure and hemostatic abnormality (Shastry *et al.*, 1977). The hematological abnormality compatible with disseminated intravascular coagulation (Mahasandana *et al.*, 1980). Thrombocytopenia and increment in fibrinolytic activity are usually observed (Sitprija *et al.*, 1974; Chugh *et al.*, 1975; Mahasandana *et al.*, 1980), and probably a consequence of intravascular coagulation (Mersky, 1976). Hypotension is not detected in the patients (Sitprija and Boonpucknavig, 1979).

The remaining electron microscopic studies deal with Russell's viper venom have been reported (Sitprija and Boonpucknavig, 1977; Aung-Khin, 1978; Date and Shastry, 1981; Date and Shastry, 1982). Sitprija and Boonpucknavig (1977) have described the electron microscopic changes in the kidneys of the patients with ARF following Russell's viper bites and illustrated glomerular changes which include mesangial proliferation, endothelial swelling and the presence of electron dense deposited in the mesangial matrix. Distal and collecting tubular epithelial cells were necrosis and regeneration. Date and Shastry (1981) have shown extensive destruction of glomerular and tubular cells with hemorrhage into the glomerular urinary space and tubules. They suggested that the presence of fibrin in the glomeruli indicated the ischemia might be due to intravascular coagulation (Aung-Khin, 1978). Date and Shastry (1982) illustrated glomerular mesangial hypercellularity,

vascular endothelial swelling, tubular epithelial necrosis and shedding, acellular segments of tubular basement membranes and the interstitial infiltrate composed of mast cells, eosinophils, lymphocytes and plasma cells. Severe vascular and tubular lesions were also presented in the medulla. They indicated the primary lesion was a reversible intravascular coagulation with secondary ischemic tubular damage and acute tubular necrosis was probably caused by a temporary interruption of tubular blood supply at the glomerular level. The nature of the interstitial inflammation is suggested a delayed hypersensitivity response to antigen that releases from damaged tubules (Seedat *et al.*, 1974; Sitprija *et al.*, 1974).

Recently, Tungthanathanich (1983) has been studied the cardiovascular and renal hemodynamics effects after administering Russell's viper venom 0.1 mg/kg.bw intravenously in dogs. A short period of 2 hours, total peripheral resistance increased by approxinately 5% whereas the renal vascular resistance increased nearly 3 folds as compared with the preinjection period. Cardiac output and stroke volume also increased during the period of 24-48 hours while renal plasma flow, renal blood flow, glomerular filtration rate, filtration fraction and urine flow rate attenuated all over the period of 48 hours. The urinary excretion and fractional excretion of sodium, potassium, chloride, calcium and inorganic phosphorus decreased after 2 hours and returned to the control level during 24-48 hours. She indicated that the tubular cell activities exhibited normal reabsorptive and secretory functions. In 1984, Tongvongchai has described cardiovascular and renal function changes

in indomethacin pretreated dogs whom compared with non pretreated dogs. Dogs without pretreated with indomethacin produced a marked reduction in mean arterial pressure and recovered to control level in a long period as compared to the other one. The percentage of increment in total peripheral resistance was dominated in indomethacin pretreated dogs, renal vascular resistance of non pretreated dogs was more increased than indomethacin pretreated dogs. Marked decrease in renal plasma flow, renal blood flow and glomerular filtration rate in non pretreated dogs. She has considered Russell's viper venom caused direct effect to produce hypotension and secondary subsequent to renal function changes so these changes may be mediated by prostaglandin.