CHAPTER II

LITERATURE REVIEW

1. General background

Snakes are reptiles classified in Class : Reptilia, Subclass : Synaptosuria, Order : Squamata, Suborder : Serpentes. All snakes have a backbone but lack limbs, eyelids and external ear openings.

Snakes have a specialized row of scales along the underside of their bodies. The bones of their upper jaws are not connected at the snout but are free to move away from one another, to allow for passage of larger prey. Food for the largest snakes may include small deer and even crocodiles. The method of capturing prey takes several forms. The boas and pythons, as well as many other species, constrict their prey, throwing several coils of their body around it and tightening their grip until the prey can not breath. Some species inject their prey with venom through fangs to immobilize them. A fang is simply a tooth modified to inject venom into prey. The fangs work in concert with other structures to form a complete venom-delivery apparatus, which function like a hypodermic syringe and needle. The venom duct does not extend into the fang. It opens adjacent to the fang, within a sheath of connective tissue surrounding the fang's base. This sheath is a seal around the fang, directing the flow of venom into the fang's canal and outward into the prey.

Snakes use some of the same senses (i.e. sight, smell, etc.) that other animals use, but they have also developed additional senses, perhaps because their eyesight and hearing are not good. Snakes use their tongues to pick up scent; particles from the atmosphere.

Temperature limits their activity and numbers in countries with cool climates. In tropical regions, snakes have moved into many habitats. Each species is well adapted to its habitat, in shape, colour and behavior, leading to a much wider variety of size, shape and colour than is generally realised. There are numerous species of venomous snakes reported worldwide (Russell, 1990).

2. Poisonous Snakes of Thailand

Around the world, there are about 2700 known species of snakes. At least 175 snake species have been identified in Thailand, of which 85 are venomous (Cox, 1991). Five families of these are venomous: Colubridae, Elapidae (cobras and kraits), Hydrophidae (sea snakes), Viperidae and Crotalidae (vipers) (Table 1). Bites by Viperids, Crotalids and Colubrids usually cause primarily local trauma and bleeding. Elapid bites most commonly cause neurological symptoms, particularly paralysis; while Hydrophid bites cause paralysis and myonecrosis (Nelson, 1989).

2.1. Family Elapidae

Elapidae snakes possess two short, permanently erect venom-conducting fangs on the anterior end of the upper jaw. They are connected to venom-producing glands.

Their pupils are round. The body is often short and relatively fat. The head is triangular in shape and there is a distinct neck.

Table 1. Classification of four snake families and sources (Bee et al., 2001)

Snake	Source
(a) Family Elapidae	
Acanthophis antarticus	Papua New Guinea
Bunagaris caeruleus	Sir Lanka
B. candidus	Thailand
B. flaviceps	Thailand
B. multicinctus	China
Dendroaspis angusticeps	Tanzania
D. Jamesoni	Nigeria
D. polylepis	South Africa
Denisonia nigrescedns	Eastern Australia
Hemachatus haemachatus	South Africa
Micropechis ikaheka	Papua new Guinea
Micrurus fulvius	USA
Naja atra	China
Naja siamensis	Thailand
Naja sumatrana	Thailand
N. kaouthia	Thailand
N. naja naja	Sri Lanka
N. nigricollis	Ghana
N. pallida	Tanzania
Ophinophagus Hannah	Thailand
Wallterinnesia aegyptia	Egypt
N. haje	Ghana
N. melanoleuca	Malawi

Snake	Source
(b) Family Viperidae	
Bitis arietans	Nigeria
B. gabonica	Ghana
B. nasicornis	Ghana
Cerastes cornutus	Saudi Arabia
Daboia russelii siamensis	Thailand
Echis coloratus	Saudi Arabia
E. pyramidum leakeyi	Kenya
E. ocellatus	Nigeria
E. sochureki	Pakistan
Vipera ammodytes meridionalis	Bulgaria
V. berus	UK
V. palaestinae	Israel
(C) Crotalinae	
Agkistodon bilineatus	Mexico
A. contortix	USA.
A. piscivorus piscivorus	USA.
Bothrops alternatus	Brazil
B. asper	Ecuador
B. atrox	Brazil
B. cotiara	Brazil
B. jararaca	Brazil
B. jararacussu	Brazil
B. moojeni	Brazil
B. neuwiedi	Brazil
B. pradoi	Brazil
B. picadoi	Brazil

Snake	Source
Bothriechis schlegelli	Costa Rica
Bothrops xanthogramma	Ecuador
Calloselasma rhodostoma	Thailand
Crotalus adamanteus	USA
C. atrox	USA
C. durissus collilineatus	Brazil
C. d. terrificus	Brazil
C. horridus	USA.
C. viridis rubber	USA.
C.v. viridis	USA.
C.v. helleri	USA.
C.v. oreganos	USA.
Hypnale hypnale	Sri Lanka
Lachesis muta	Brazil
Porthidium nummifer	Costa Rica
P. ophryomegas	Costa Rica
Sistrurus catenatus	USA.
Trimeresurus albolabris	Thailand
T. flavoviridis	Japan
T. macrops	Thailand
T. popeorum	Thailand
T. purpureomaculatus	Thailand
T. stejnegeri	Thailand
T. sumatranus	Sumatra

Snake	Source
(d) Hydrophidae	
Enhydrina schistose	Australia
Hydrophis cyanocinctus	Malaysia
H. spiralis	Malaysia
(e) Colubridae	
Bolga dendrophla	Thailand
Rhabdophis subminatus	Malaysia

2.1.1. Genus Naja, the Cobras

All Asiatic cobra populations are regarded as belonging to one single genus, *Naja naja*. (Table 2) (Wuster, 1995).

2.1.1.1. Naja kaouthia (Monocellate Cobra)

Identification : Up to 2 meters long. Hood markings usually shaped like ocelots or a mask. Color and pattern variable, ranging from black to light brown. Albinos with red eyes are not uncommon. Many specimens are without body markings but can also be banded or speckled (Cox, 1991).

Ecology : Encountered during both day and night in termite mounds, near human habitation, in forests, and in foothills up to 900 meters. The first defense is to flee. If threatened, snake will raise the anterior third of its body, expand its hood, usually hiss, and strike if the predator comes too near. They may strike with closed mouth but may also bite and hold on with a chewing motion.

Range : Throughout the country. Common in the central plain and the southeast but also seen in the north and northeast.

Extralimital : Northeastern India, Andaman Island, Nepal, Bangladesh, Burma, Malaysia, Laos, Cambodia, southern Vietnam, and southern China (Cox, 1991).

2.1.1.2. Naja siamensis (Indochinese Spitting Cobra)

Identification : Up to 160 cm in length, usually 90 to 130 cm. Hood markings are spectacle, U-, V- or H-shaped, and often absent. Color and patterns highly variable. Some specimens may be predominantly black, or mottled with black and white spots, or even predominantly white. Some specimens are olive-green or grayish-green (Cox, 1991; Wuster and Trope, 1994).

Remarks : The fangs have anterior openings, enabling the "spitting" of venom for a distance of up to 2 meters. It is the primary means of defense. When venom enters the eyes, a burning sensation is immediately felt. Spitting cobras also bite readily.

Ecology : Found up to 500 meters in forests and on agricultural land, where it hunts for rodents and other small mammals. Encountered during both night and day.

Range : A rather common snake in the western and central regions, especially in the provinces of Ang Thong, Suphan Buri, Kanchanaburi, and Tak. It is also found in the southeast and as far south as Phetchaburi.

Extralimital : It is often confused with *Naja kaouthia*, the complete range is not yet defined.

2.1.1.3. Naja sumatrana (Equatorial Spitting Cobra)

Identification : Usually 90 – 120 cm in length, rarely 150 cm or more. The patterns are no hood mark; highly variable, many differences between populations. Juveniles have a light throat area with one or several pairs of lateral spots and often a median spot, in adults, black pigment usually obscures most of throat area; occasionally, some white cross-bands in juveniles.

Ecology : Equatorial south-east Asia.

Range : Southern Thailand.

Extralimital : Malaysia, Indonesia (Sumatra, Borneo, Bangka, Belitung, the Riau Archipelago) and Philippines (Palawan, Culion); may occur on other islands in the region, possible remnant population in western Java.

2.1.2. Genus Ophiophagus , the King Cobra

Head distinct from neck, pupils round. The hood is narrow and long and is spread when disturbed.

***2.1.2.1. Ophiophagus hannah* (King Cobra)**

Identification : Average length , 4 meters and up to 5.85 meters. The hood is longer and narrrower than *Naja* but without pattern. A pair of large occipital scales are behind the parietals; these together with the hood shape, serve as identifying characteristics. Newborn specimens are black and have light yellow bands. Some adults are light green or orange – yellow and maintain a hint of the juvenile pattern.

Ecology : Encountered during both day and night, terrestrial, most often found in forests and plantations, up to 2,135 meters. King cobras feed only on snakes and lizards. They

prepare a nest and lay 21 to 40 eggs, which the female guards. They usually hiss loudly before striking

Range : Found throughout Thailand.

Extramilital : India through mainland southeast Asia into Indonesia, the Philipines , and southern China.

Table 2. Classification of older names used in the literature for the populations of Asiatic cobra. (Wuster, 1995)

Old name	Current name of species , and populations for which (mis)used
N. atra	*N. atra* (China, Taiwan , northern Vietnam), *N. siamesis* (Thailand)
N. isanensis	*N. siamesis*
N. Naja	All asiatic cobras
N. kaouthia	*N. kaouthia, N. sagittifer* (Andamans)
N. Kaouthia suphanensis	*N. kaouthia* (Thailand, rare)
N. n. atra	*N. atra* (China, Taiwan , northern Vietnam), *N. siamesis* (Thailand)
N. n. caeca	*N. oxiana , N. naja* (patternless specimens from northern India)
N. n. indusi	*N. naja* (NW India , Pakistan)
N. n. isanensis	***N. siamensis***
N. n. kaouthia	*N. kaouthia, N. siamesis* (through confusion*), N. sagitifera* (Andaman Island), N. *sumatrana* (northeran Malaysia –Reid, 1964; Tweedie, 1983)
N. n. karachiensis	*N. naja* (southern Pakistan black form)
N. n. leucodira	*N. n. sumatrana* (Malayan Peninsula, Sumatra), *N. kaouthia* (Reid, 1964)
N. n. miolepis	*N. sumatrana* (Borneo)
N. n. naja	*N. naja*
N. n. oxiana	*N. oxiana , N. naja* (patternless specimens from northern India)
N. n. philippinensis	*N. philippinensis*
N. n. polyocellata	*N. naja* (Sri Lanka, rare)
N. n. sagittifera	*N. sagittifera*
N. n. samarensis	*N. samarensis*
N. n. siamensis	Probably *N. kaouthia* (in the toxinological literature)
N. n. sputatrix	*N. sputatrix* (Java, Lesser Sunda Islands), *N. sumatrana* (Malayan Peninsula, Bangka, Belitung), *N. siamensis* (Thailand), *N. kaouthia* (Vietnam, rare)

Old name	Current name of species , and populations for which (mis)used
N. n. sumatrana	*N. sumatrana* (Sumatra)
N. n. sputatrix atra	*N. siamensis* (Thailand), N. atra [China, Taiwan, northern Vietnam (Lingenhöle and Trutnau, 1989)]
N. sputatrix isanensis	*N. siamensis* (Thailand)
N. sputatrix sputatrix	*N. sumatrana* [Malayan Peninsula, Java (Lingenhöle and Trutnau, 1989)]

2.1.3. Genus Bungarus

Head is not distinct from neck , scales smooth , eyes small with round pupils.

2.1.3.1. Bungarus fasciatus (Banded Krait)

Identification : Up to 2 meters in length. Body stocky, vertebral ridge very distinct, tail blunt. Yellow and black bands of almost equal width alternate and encircle the body and tail (Cox, 1991). Head predominantly black .

Ecology : Noctural and terrestrial. Common in lowlands but recorded at 2,300 meters. Prefers dry places. Found in open country, forests, and fields. Feeds on cold-blooded animals. It is shy and inoffensive during the day, often hiding head under the body when approached, but bites readily without warning at night. Banded kraits may venture into human dwellings.

Range : Throughout Thailand.

Extramilital : Northeastern India, Burma, Cambodia, Laos, Vietnam, southern China, both west and east Malaysia, and Indonesia.

2.1.3.2. Bungarus candidus (Malayan Krait)

Identification : Up to 1.44 meters in length. Tail pointed. A series of black and white bands alternate over the body. Black bands do not encircle the body.

Ecology : Nocturnal and terrestrial. Malayan kraits bite readily at all times without hissing. They feed on cold – blooded animals.

Range : Throughout Thailand.

Extramilital : Burma, Malaysia, Cambodia, and southern Vietnam.

2.1.3.3. Bungarus flaviceps (Red Headed Krait)

Identification: The red–headed Krait maintains a head and tail of what is described in folklore like that of a pigeon blood ruby. With a steely black/blue ground color with a low lateral white stripe running the entire length. Snake eaters for the most part, and easily switched to mammals. They are fast and unpredictable

Ecology : Nocturnal and terrestrial.

Range : Throughout Thailand.

Extramilital : Burma, Cambodia and Vietnam

2.2. Family Viperidae

The viperid snakes are divided into two groups; the pit or crotaline vipers which have a thermosensitive pit between the eye and the nostril, and the pitless vipers. The pit vipers have triangular shaped heads, elliptical pupils, and a single row of subcaudal scales distal to the anal plate. Each member of this family has a pair of large, hollow fangs located anteriorly on the upper jaw that are connected by a duct to a venom-producing gland. The fangs fold back against the roof of the mouth when it is closed. They swing forward to deliver a strike.

2.2.1. Subfamily Crotaline

These snakes have a prominent pit located between the nostril and the eye that leads to a thermosensitive organ used in finding prey.

2.2.1.1. Calloselama rhodostoma (Malayan pit viper)

Identification : Malayan pit viper is a medium size venomous snake, has a thick body and grows to only about 1 meter long, with a short tail. Head triangular, snout turned up to the tip, with large scales. Dorsal surface has dark triangular markings on a reddish or purplish-brown background. Venter whitish-brown and mottled.

Ecology : Prefers less humid, sandy, forested lowlands up to 2,000 meters. Often encountered on rubber plantations. The Malayan pit viper is noctural in habit and prey on mice and frog. It is oviparous and lays around 13 to 30 eggs. When threatened, it remains motionless and relies on camouflage to avoid detection. If the threat approaches

too near, it will strike quickly without hissing. It is an occupational hazard of rubber plantation workers and farmers because it is poorly visible.

Range : Throughout Thailand except for extreme high – lands.

Extramilital : Malaysia , Indonesia, Laos, Vietnam and Cambodia

2.2.1.2. Trimeresurus albolabris (White-Lipped Pit viper)

Identification : *Trimeresurus albolabris* is a small snake. The longest size that was found is 1 meter. The head is long-triangular shape and is obviously bigger than the neck. Mostly, this snake has a green head, green body, yellow ventral, yellow or white lip, red tail, and yellow or red eyes. The scales on this snake's head are rather smooth and small. It is a fierce snake and quickly bites.

Ecology : It is nocturnal and always looks for its prey on a tree, in a branch or on the ground. Its food is mice, bird and lizard.

Range : This viper can be found in all parts of Thailand and most often found in Ayutthaya, Nakorn Pathom and especially in Bangkok. It always lives near human habitation.

Extramilital : It is also found in India, Bangladesh, Southern China, Malaysia, and Indonesia.

2.2.2. Subfamily Viperinae

These snakes do not have a pit located between the nostril and the eye.

2.2.2.1. Daboia russelli siamensis (Siamese Russell's viper)

Identification : Up to 1.5 meter long. Head triangular, short, rounded snout with protruding upper edge, and large nostrils. Body light brown with multicolored blotches. The blotches vary in shape and size; all, however, are dark brown with a black inner edge and a white outer edge. It usually feeds on small preys, such as mice, rats, birds, etc.

Ecology : Avoids dense vegetation and can be found between rocks, in earth cavities , or in old termite mounds. Many enter human dwellings. Remains motionless and relies on camouflage to avoid detection. Normally, this snake moves very slowly but quickly strike in every direction when defending. When it is scared, the body will be a circle and make a noise to threat an enemy.

Range : This snake has been recorded within Bangkok and nearby provinces, at Lop Buri, Saraburi, Chai Nat, and Nakhon Ratchasima; it may also be present in Amphoe Thap Sakae of Prachuap Khiri Khan province in the south.

Extralimital : Burma, Cambodia, Vietnam, and southern China. Subspecies are reported in India, Pakistan, Bangladesh, Sri Lanka, Indonesia, and Taiwan.

2.3. Family Colubridae

These snakes have enlarged, solid fangs on the posterior maxillary bone referred to as back – fanged. One such species is found in Thailand. Although the vast majority of

members of this family are not a danger to humans, a few produce venom that has proven fatal.

2.3.1. Rhabdophis subministus (Red – Necked Keelback)

Identification : Up to 1.3 meter in length. Head distinct from neck. The supralabials are gray, but the infralabials are white. The neck is reddish and the body is uniformly olive – green .

Ecology : Semiaquatic place.

Range : It is found throughout Thailand in wet forests up to 1,780 meter. The venom action of this snake is still largely unstudied.

2.4. Family Hydrophidae

Characterized by vertically flattened, oar–shaped tails and very small ventral scales. They have fixed, needle–like anterior fangs. Entirely marine but occasionally seen several kilometers within rivers. There are many species in the sea around southeast Asia. *Enhydrina schistosa* venom is among the most potent known, and one "drop" (0.03 ml) can kill three humans (Reid and Tu, 1987 ; McCarthy and Warrell, 1991; Vick, 1994; Higa, Uezato and Araki, 1990).

2.4.1. Enhydrina schistosa (Beaked Sea Snake)

Identification : Up to 1.4 meter in length, head small. The body is thick. Skin appears loose.The youngs are gray or bluish–gray dorsal, whitish ventral surface with dark gray or black rings that usually fade with maturity.

Range : Not common but recorded off both coasts of Thailand.

Extralimital : *E. schistosa* ranges widely throughout the warm Pacific Ocean and has inflicted bites in Malaysia and Australia.

3. Epidemiology of snakebite in Thailand

Snakebite is an important public health problem throughout the world, especially in rural areas of tropical and subtropical countries (Reid and Theakston, 1983). It is estimated that the true incidence of snake envenomation could exceed 5 million cases per year. About 100,000 of these develop severe sequelae because of the diversity of economic and ecological condition (Bhetwal, O'Shea and Warrel, 1998). In Thailand, approximately 10,000 venomous snakebites occur each year. An estimated 2,000 of these bites in which the poisonous snakes were identified. In the 1940s, more than 200 deaths were reported each year (Swaroop and Grap, 1954), but the figure declined to 80 a year in the 1960s (Trishnananda et al., 1979) and to less than 20 a year from the 1980s to the 1990s (Division of Epidemiology, 1982-1999). The Queen Saovabha Memorial Institute (Thai Red Cross Society) has produced specific monovalent antivenoms since 1923 (Purananda, 1956). This has been purified and pepsin digested since 1968.

The top ten provinces having the highest bite rates per 100,000 population were Prachin Buri (70.94), Chai Nat (66.92), Prachuap Khiri Khan (64.39), Uthai Thani (58.49) Ang Thong (58.27), Pathum Thani (52.61), Nakhon Sawan (52.29), Ratchaburi (51.19), Trang (50.70) , and Sing Buri (48.43) (Divisionof Epidemiology, 1999).

The majority of deaths occur among children and the elderly and among those for whom activenom has not been given, has been postponed, or has been administered

in insufficient quantities; and among members of fundamentalist religious groups who handle poisonous snakes during their religious rituals (Russell and Banner, 1988). The majority of the victims were male, 25 – 35 years of age. More than 80 % of the bites were inflicted on the extremities (feet and ankles) of agricultural workers, herders and hunters who inadvertently step on a snake. This suggest that in most cases the snake is inadvertently trodden upon. The peak months of bites occurred between May–June and September – October (Division of Epidemiology, 1995-1999). There is a marked seasonal variation in the incidence of snake bite; peaks being associated with the rainy season or with increased human and snake activity. Heavy flooding may cause epidemics of snake bite by concentrating human and snake populations in small areas of dry ground.

Morbidity and mortality resulting from snakebite envenomation also depend on the species of the snake involved. Four species (*Naja kaouthia, Calloselasma rhodostoma* , *V. russelli siamensis* , *Bungarus candidus*) are responsible for fatal snake bites. *Naja kaouthia* was responsible for 25 % of 4850 cases of snakebite reported in Thailand during a five year period with cases fatality of 6.5 % (Trishnananda et al., 1979, Looareesuwan, Viranvan and Warrell, 1988). In all cases, respiratory failure was the cause of death , with complicating shock, septicaemia and renal failure. In addition to the usual neurotoxic signs, patients had local swelling with or without blistering and necrosis. *Calloselasma rhodostoma* (*C. rhodostoma*) inflicts permanent local injuries on many rubber plantation workers in South–East Asia but bites are rarely fatal (Warrell et al., 1986). *C. rhodostoma* is particularly abundant in the eastern and southern provinces but has been collected in all provinces (D.A. Warrell, personal

communication). Without specific treatment, the mortality of *C. rhodostoma* bite is on 1% - 2% (Reid et al., 1963, Looareesuwan, Viravan and Warrell, 1988). These patients developed gangrenous extremities leading to death from tetanus, septicaemia and spontaneous systemic bleeding. The large number of individuals bitten by *C. rhodostoma* and the case with which it can be killed and brought for identification, have made it possible to conduct large scale studies of proved *C. rhodostoma* envenomation. In the central rice–growing area of Thailand, *B. russelli siamensis* is the main culprit (Kanjanajatanee and Visutipant , 1984) and still causes more than 1000 deaths per year in neighbouring Burma (Mynt – Lwin et al., 1985). *Bungarus fasciatus* is an unusually innocuous species which causes very few bites. However, one fatal case was detected by immunodiagnosis in a group of suspected cobra bites (Viravan et al., 1986). *Bungarus candidus* was responsible for as many deaths as *C. rhodostoma* and *Naja kaouthia. Bungarus candidus* is also a problem in Vietnam (Pearn, 1971) and Java (Kopstein, 1930) where deaths have been also caused by this species. Its cryptic noctural habits (Taylor, 1965) have led to being regarded as a rare snake in Thailand (Smith, 1923) but it is well known by the Thai name, *ngoo tapsamingklaa*, in rural areas of eastern and southern Thailand. Bungarus bites are almost painless leading to delay in seeking medical care. In Malaysia it was also said to be well distributed but rather rare (Tweedie, 1983).

4. Snake venom

Snake venoms are evolutionary adaptations to immobilize prey and as secondary use in defense. Venoms are highly toxic secretions produced in special oral glands and

can be considered a modified saliva. It is produced by a pair of large venom glands. One gland is located on each side of the head, below and behind the eye and above the upper rear corner of the jaw. Within these glands, which are typically almond–or pear–shaped, the venom is produced by several (usually four to five) lobes of secretory cells. The secretory cells can make up as much as 80 % of the gland's total cell content. Activation of the secretary epithelium in these glands induces and accelerates the rate of protein synthesis. Their secretions drain through small tubules into a hollow space, the lumen of the gland. The lumen joins the venom duct, which carries the venom forward to the base of the fang. The venom duct is surrounded by small masses of glandular tissue, the accessory glands, which may act as valves to regulate the flow of venom to the fangs (Seiler et al., 1994).

4.1. Venom composition

Venom components will vary not only between species, but within the same species depending on geography, season, age and nutritional status of the snake (Holstege et al., 1997). Venoms usually contain about 80 –90% water and approximately 90% of the dry weight of most venom consists of protein material. (Walter et al., 1999). Most of the toxic and biologically active components of the venom are proteins and form the main fraction of the dry weight. The nonprotein fractions are metal ions, inorganic anion and some small organic molecules including peptides, lipids, nucleosides, carbohydrates and amines (Barbara and Hawgood , 1998). A snake venom contains many different proteins, some are highly toxic, some are nontoxic. Toxicity of snake venom is due to a combined effect of all components.

Snake venoms are composed of several series of fractions (Chippaux and Goyffon, 1998).

- Toxins affecting the neuro-muscular system, the most important of which are the presynaptic neurotoxins that often possess phospholipase activity and the postsynaptic curare–like neurotoxins.
- Toxins which bind cell membrane receptors inducing cytolysis.
- Hemorrhagins, that cause damage to the vascular endothelium.
- Factors acting on blood coagulation, numerous, but largely dominated by the thrombin – like enzymes that convert fibrinogen in fibrinopeptides.
- Enzymes, possessing various structures and activities but showing generally a reduced toxicity as compared to that of neurotoxins.

4.1.1. Toxins.

Toxins are proteins of various molecular weights. They have target specific receptors, mostly on cell membranes. The specificity of toxins can be neurological, cardiovascular, muscular or not differentiated according to the anatomical distribution of recognized receptors. Neurotoxins are more toxic than others because they are lethal. Snake neurotoxins are peripheral neurotoxins, rather than central active neurotoxic. There are several types of neurotoxins and their structures, the site of action and the mechanism are not identical.

4.1.1.1. Postsynaptic Toxins.

Postsynaptic neurotoxins are commonly found in the venoms of Elapidae and Hydrophiidae. The toxins affect the neuromuscular junction at the postsynaptic site by combining with nicotinic acetylcholine receptor (AchR). These neurotoxins act on the muscle side, rather than the nerve side. The so–labeled *postsynaptic neurotoxins* are affect muscle contracture and should not have been designated neurotoxins. They bind to the acetylcholine receptor in the muscle that is to receive the neurotransmitter acetylcholine. On the other hand, the attachment of acetylcholine to the acetylcholine receptor is considered a part of the nerve–transmitter mechanism. The paralysis of the muscle by postsynaptic neurotoxin poisoning is essentially due to the formation of an AchR neurotoxin complex.

The structure of postsynaptic neurotoxins is well studied. There are actually two types of these neurotoxins. One type has four disulfide bonds (Called type I or short–chain neurotoxins) while the other type has 5 disulfide bonds (called long-chain neurotoxins). The short–chain neurotoxin has one or two amino acids in segment 8, whereas the long–chain neurotoxins have a longer segment 8. Another difference is that there is only one amino acid within segment 5 of the short chain neurotoxin, whereas the long–chain neurotoxin has three amino acid residues within the segment (Tu, 1998).

4.1.1.2. Presynaptic neurotoxins.

The presynaptic neurotoxins are also called β-toxins in contrast to postsynaptic or α-toxins. When a β-toxins is added to the neuromuscular preparation, the muscle contraction starts without stimulation of the nerve axon. β-toxin usually does not affect

the depolarization of the muscle itself nor has a binding ability to the acetylcholine receptor. It is thus clear that the β-toxin somehow affects the presynaptic end of the nerve and initiates the release of acetylcholine and then eventually stop the release.

The most typical toxin of this type is β-bungarotoxin. It consists of two chains: The A chain has 120 amino acid residues and the B chain has 60 amino acid residues. The amino acid sequence of the A chain is similar to the phospholipase A_2 sequence and, in fact, the A chain does possess phospholipase A_2 activity (Hendon and Tu, 1979).

Presynaptic neurotoxins are found principally in kraits (β- bungarotoxin), some Australian elapids (notexin, taipoxin, textilotoxin) and a few vipers (crotoxin) (Bon et al., 1979).

4.1.1.3. **Cardiotoxins** .

Cardiotoxins stop the heartbeat when they make contact with the heart. Cardiotoxins have four disulfide bonds and a very short segment 8. In this manner, they are similar to short–chain postsynaptic neurotoxins. Although the similarity in disulfide bonds and the peptide backbone is remarkable for cardiotoxins and postsynaptic neurotoxins, there are considerable differences between them in amino–acid composition and sequences. Carditoxins do not bind to the AChR, whereas there is strong binding between the postsynaptic neurotoxins and the AChR. The hydrophilic index of carditoxins shows them to be quite hydrophobic molecules, whereas the neurotoxins are quiet hydrophilic molecules. They are more general toxins, affecting cell membranes, whereas neurotoxins are specific toxins, binding to acethycholine receptors (Tu, 1998).

4.1.1.4. Pharmacological action of neurotoxins

4.1.1.4.1. Systemic envenomation.

The characteristic systemic signs in these victims result from neuromuscular involvement. The onset of various neuromuscular symptoms are in four clinical groups.

a) The first sign was drowsiness and ptosis.

b) The second signs are flaccid skeletal muscle or general weakness followed by respiratory paralysis, palatal paralysis, and glossopharyngeal paralysis.

c) The third signs, breathing difficulties, dypnea and apnea were the usual manifestation.

d) The fourth sign is coma

The preparalytic symptoms are frequently absent and vomitting, loss of consciousness, headache, vasomotor sign (pallor , sweating, weak to absent pulse and hypotension) are also seen.

- **Cardiotoxicity** : Cardiotoxins cause increase in blood pressure and increased cardiac output. This is followed by myocardial depression and asystole. Mortality approaches 100% if cardiotoxic complications occur, especially from Taiwan cobra venoms.

4.1.1.4.2. Local envenomation .

Local tissue necrosis is now accepted as the most common feature of bites by some cobras such as *Naja kaouthia.* Cardiotoxins, also known as cytotoxins and direct lytic factors, and myotoxins, are particularly common in the venoms of cobras, and are almost certainly responsible for the myonecrosis. After cobra bites, a dusky

discoloration around the bite marks extends and develops deepening colour. After 3-4 days a greyish black area becomes encircled by a red raised rim and sanguineous blisters may occur. Fluctuation later becomes apparent, and necrosis of skin and subcutaneous tissue is usually evident by 5^{th} day. The area of skin necrosis may vary from a few square centimeter up to 600 cm^2. The extensive skin loss may take several months to heal. Necrosis of skin and subcutaneous tissue was frequently so extensive that skin grafting was required, and in some cases a certain degree of disfigurement persisted. The incidence of subsequent local necrosis in these patients was quiet high, about 40% of the total cases. (Mitrakul et al., 1984).

4.1.2. Enzymes.

Venom enzymes whose molecular weights are generally higher than those of toxins. Their catalytic properties, which distinguish them from toxins, have two major consequences. The product of degradation, even if toxic have, no immunogenic properties. Pharmacological effects depend more on the time in the enzymatic reaction cycle than of the initial quality of enzymes. In general enzymes in snake venoms act in the following ways .

a) cause local capillary damage and tissue necrosis.

b) induce acute hypotension and pain.

c) cause diverse coagulation and anticoagulant actions.

Viperidae and Crotalidae venoms contain more enzymes than cobra venoms. Venom proteins have many diverse enzymatic activities including phospholipase A_2, phosphodiesterase, phosphomonoesterase, L-amino acid oxidase, acetylcholinesterase,

arginine esterase, 5' nucleotidase, hyaluronidase and nicotinamide–adenine dinucleotide, nucleosidase and proteolytic activities (Stocker, 1990). Metalloproteinases (Bjarnason and Fox, 1994) and phospholipase A_2 (Gutierrez and Lomonte, 1997) are the main enzymes for inducing hemorrhage, edema and tissue necrosis in victims.

4.1.2.1. Metalloproteinase .

Proteases can be classified according to their specificity. The classification schematic most widely used is based on structural similarities within the active sites of proteases. The proteases are named for a particular amino acid that plays a key structural and functional role in the active site. At present, three types have been described.

a) serine protease

b) cysteine protease

c) metalloproteinase (contain an atom of zinc in their active site).

Metalloproteinases are one of several classes of proteolytic enzymes (Neurath, 1989) which are produced by a variety of bacteria (Jiang and Bond, 1992) and venomous snakes (Bjarnason and Fox, 1995; Kini and Evans, 1992; Bjarnason and Tu, 1978). They consist almost entirely of zinc compounds and are containing from 20,000 to 10,000 mol. wt. The zinc content was determined only on a few of them (Nikai et al., 1982, 1984; Mori et al., 1987; Daoud et al., 1987). Zinc metalloproteinases are members of the metzincin family (Stocker and Bode , 1995). Members of this family are so named by virtue of their being zinc containing metalloproteinases and having a

common methionine turn below and carboxy-terminal to a helical segment containing two of three histidine residues involved in the zinc-binding site. The methionine turn forms a hydrophobic basement beneath the central active site helix and the substrate binding cavity. Members of this family include mammalian matrix-degradating metalloproteinases (the matrixins), bacterial metalloproteinases (the serralysins), as well as the venom metalloproteinases (adamalysins). The zinc binding site has a common amino acid sequence in the different members of this family of metalloproteinases, HEBXHBGBXHZ , where H is histidine, E is glutamic acid, G is glycine, B is bulky hydrophobic residue, X is any amino acid, and Z is different in all four subfamilies but is conserved in any given subfamily. Adamalysin is a 24 Kda metalloproteinase that contains one zinc and one calcium atom per molecule.

On the basis of their domain structure, snake venom metalloproteinases have been classified in four main groups (Hite et al., 1994).

1) P-I, comprising only the metalloproteinase domain
2) P-II, having a metalloproteinase domain followed by a disintegrin-like domain
3) P-III, comprising metalloproteinase, disintegrin-like and high-cysteine domains.
4) P-IV, a group of enzymes that present, besides the three described domains, an additional lectin like polypeptide linked by a disulfide bridge to the metalloproteinase-containing polypeptide chain.

Snake venom metalloproteinases play a key role in the prominent local tissue damage characteristic of viperine and crotaline snake envenomations, and also in

the systemic alterations characteristic of this pathology. Metalloproteinases induce hemorrhage, myonecrosis , skin damage and inflammation, activating endogenous MMPs and releasing a variety of inflammatory mediators (Figure 1) which are intimately involved in metastasis (Vassalli and Pepper, 1994). In addition, metalloproteinases degrade extracellular matrix components and impair the regeneration of affected skeletal muscle. Some of them also affect platelet function, through their disintegrin-like domain, and degrade blood clotting factor, precluding a normal hemostatic response after microvessel damage (Seegers and Ouyang et al., 1979; Ouyang, Teng and Huang, 1992; Hutton and Warrell, 1993; Kamiguti and Sano-Martins, 1995).

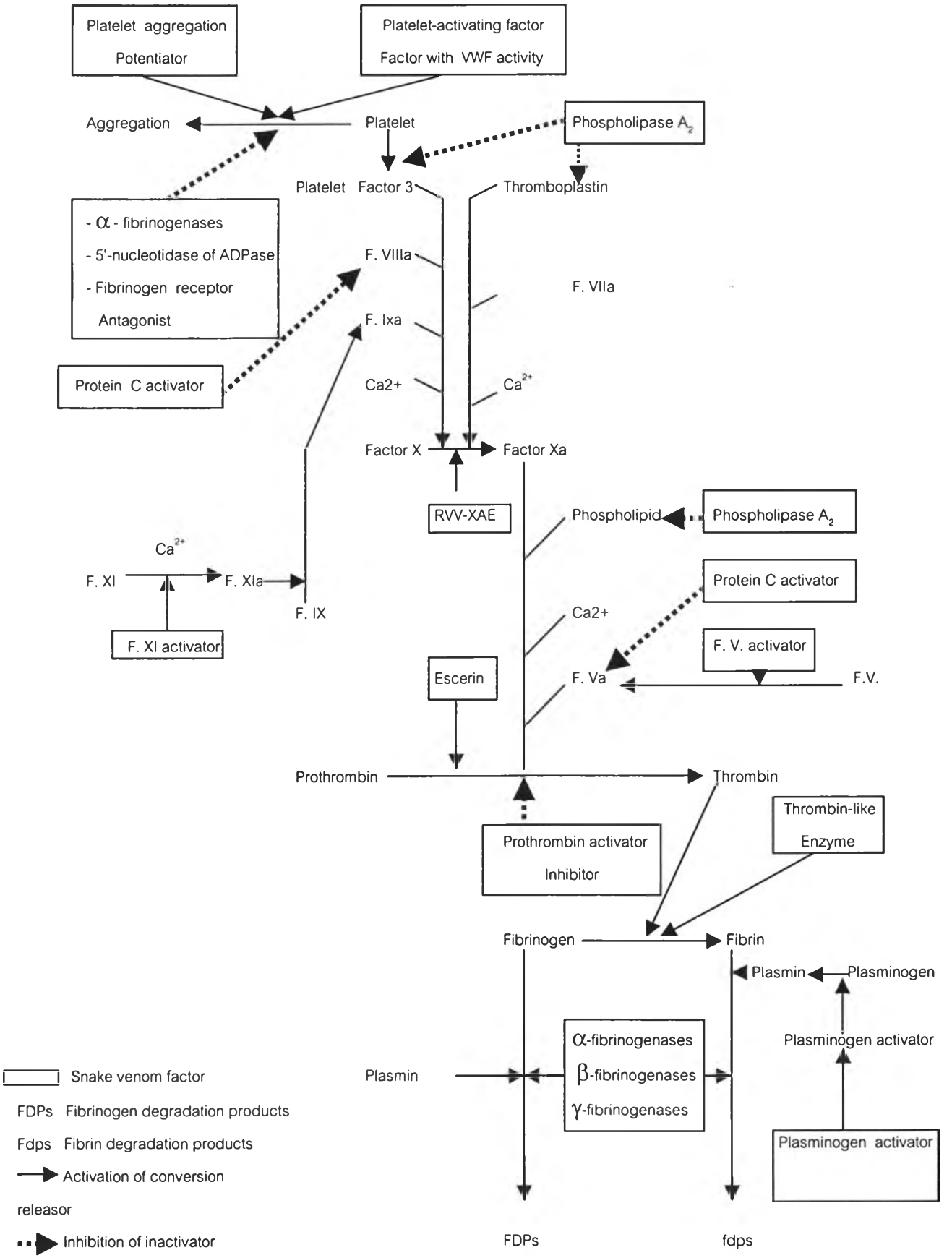

Figure 1. Action Mechanisms of snake venom factors affecting blood coagulation and platelet function. (Ouyang, Teng and Huang, 1992).

4.1.2.1.1. Pharmacological actions of metalloproteinase

- *Induction of local haemorrhage*

Hemorrhagic metalloproteinases from crotalid and viperid venoms produce local bleeding by causing lesions in the walls of small blood vessels (Ohsaka, Just and Habemann, 1973; Ownby, 1990). It is believed that this is caused by proteolysis of components of the basal lamina of the microvasculature (Ohasaka , Just and Habemann, 1973; Bjarnason and Tu, 1978; Bjarnason and Fox, 1988). The studies of their substrates have dmonstrated that these enzymes degrade all major proteins of the extracellular matrix (ECM) (Bjarnason and Fox, 1988,1989). Thus, a positive correlation exists between proteolytic activity of metalloproteinases and their haemorrhagic potencies. (Bjarnason , Hamilton and Fox, 1988; Bjarnason and Fox, 1988, 1989).

Two mechanisms by which erythrocytes and blood components escape from the blood vessels damaged by haemorrhagic toxins and enter the tissue compartments have been described: one is haemorrhage *per diapedesis*, through widened junctions between endothelial cells, the other is *per rhexis*, through gaps within the damaged endothelial cells (Ownby, 1990; Lomonte, 1994).

- *Systemic effect on blood components* (Figure 2)

The majority of metalloproteinases are also known to hydrolyse fribrinogen and since fibrinogen is an important cofactor in platelet aggregation (Hawiger et al., 1982; Plow et al., 1984), it has been proposed that the inhibition of platelet aggregation caused by these enzymes is due to degradation of

fibrinogen (Teng and Huang, 1991; Ouyang, Teng and Huang, 1992). Metalloproteinase could interfere with platelet function in two ways: first, by degrading different platelet receptors and adhesive proteins involved haemostasis and, second, by a non-enzymatic (disintegrin-mediated) interference with the function of platelet adhesion receptors.

I) Effects of metalloproteinase on platelet surface proteins

The major platelet receptors for macromolecular ligands involved in adhesion and aggregation are gpIb (von Willebrand Factor and thrombin receptor), $\alpha_{IIb}\beta_3$ integrin or gpIIb/IIIb (receptor for fibrinogen and several other RGD-containing ligands), $\alpha_2\beta_1$ integrin (collagen receptor) and gpIV (thrombospondin receptor). Of these receptors, gpIb is highly susceptible to proteolysis by both endogenous and exogenous proteinases (Cooper et al., 1982; Wicki and Clemetson, 1985). This is because the exposed extracellular region of gbIb, known as glycocalicin, is a good substrate for different enzymes.

II)Effect on plasma proteins involved in haemostasis

Venom metalloproteinases are also known to digest fibrinogen and are therefore referred to as either α or β-fibrinogenases, depending on whether the enzymes preferentially degrades the Aα or Bβ chains of the fibrinogen molecule (Markland, 1991). The majority of haemorrhagic metalloproteinases are α-fibrinogenases. Because some venom α-fibrinogenases have also been shown to inhibit platelet aggregation, their inhibitory activity on platelets was attributed

to fibrinogen degradation (Ouyang, Teng and Huang, 1987,1992; Teng and Huang, 1991). Whether β -fibrinogenases of this group of enzymes have any effects on platelet function has never been investigated.

III)Proteolysis of von Willebrand factor (vWf)

VWf plays important role in haemostasis following vascular injury by first binding to the subendothelium and then to platelets gpIb (Baumgartner, Tschoop and Weiss, 1978; Weiss, Turitto and Baumgartner, 1986). In plasma, vWf is found in the form of disulphide-linked multimers of different size ranging from 500,000 > 20,000,000 mol.wt. Each native vWF binds to platelet gpIb, and one binding site for platelet $\alpha_{IIb}\beta_3$ integrin containing the RGDS (Titani et al., 1986). Metalloproteinases cleave a part of the vWF molecule (A1 domain) which contains the site responsible for vWF binding to platelet gpIb. The proteolysis of vWF in conjunction with its effects on platelet-collagen interaction can impair the first step of the haemostatic process .

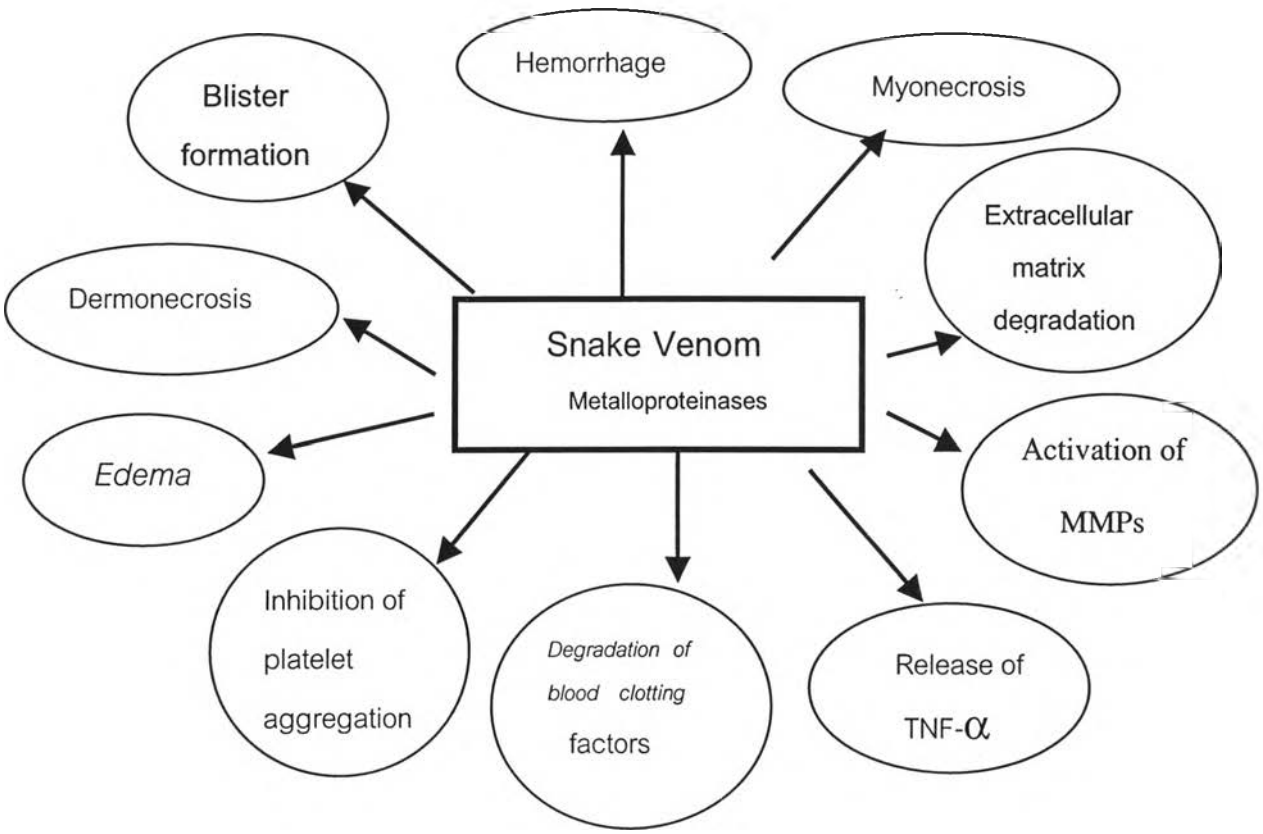

Figure 2. Summary of the multiple roles played by snake venom metalloproteinases in the pathogenesis of local tissue damage. Some effects are due to the direct action of venom metalloproteinases, whereas others are indirect effects and develop as a consequence of the action of these enzymes. Inhibition of platelet and degradation of blood clotting factors constitute systemic alterations and play a role in systemic bleeding, but they also contribute to local tissue damage, probably by potentiating the hemorrhage effect resulting from the action of metalloproteinases in the local microvasculature (Kini and Evans, 1989).

4.1.2.2. Phospholipase A_2

Phospholipase A_2's (PLA_2) are relatively small (mol. wt. about 14,000) and fairly homologous enzymes which fold mainly into three larger α-helices and a short two-stranded β-sheet. They are usually cross-linked by seven disulfide bridges which stabilize the polypeptide chain. The disulfide bridge between residues 11 and 77 is found only among Elapidae (cobras) Hydrophidae (sea snakes) and mammalian pancreatic sources, whereas that between residues 50 and 133 is found only among Crotalidae, Viperidae and the human synovial fluid. (Heinrikson, Krueger and Kein, 1977; Kramer et al., 1989).

The phospholipase A_2 superfamily consists of a broad range of enzymes defined by their ability to catalyze specifically the hydrolysis of the center (*sn–2*) ester bond of substrate phospholipids (Davidson and Dennis , 1990 ; Dennis, 1994, 1997; Balsinde and Balboa, 1999). The hydrolysis products of the PLA_2 reaction are free fatty acid and lysophospholipid. The fatty acids released by PLA_2, such as arachidonic acid (AA) and oleic acid (OA), can be important as stores of energy, but more importantly AA can also function as a second messenger (Gijon and Leslie, 1999; Berk and Stump, 1999) and as the precursor of eicosanoids, which are potent mediators of inflammation and signal transduction (Austin and Funk, 1999; Devillier, Baccard and Advenier, 1999; Bingham and Austen, 1999). The other product of PLA_2 action, lysophospholipid, is important in cell signaling, phospholipid remodeling, and membrane pertubation (Figure 3) (Moolenaar et al., 1997; Balsinde , Balboa and Dennis, 1997). The enzyme activity was first studied in phenomenological detail as early as the 1890's using "poison" or venom from cobras. A secreted PLA_2 with similar properties was found in

large amounts in porcine pancreas. With the abundant number of closely related PLA_2's from snake venoms and mammalian secretions that fall into Group I, II, V, X, the similarities of these enzymes will be described, followed by the specific differences and important characteristics for each Subgroup, and then the Group III, IX, and XI PLA_2 will be considered (Table 3). The Group I, II, V, and X PLA2's are very closely related, and share a common mechanism for cleavages of the *sn-2* ester bond of phospholipids. Hydrolysis proceeds through the activation and orientation of a water molecule by hydrogen bonding to the active site histidine, which dictates the pH dependence of 7-9 for all PLA_2's with a catalytic histidine, fall into Groups I, II, III, V, IX , X, and XI and shall be referred to as the Histidine PLA_2' s for convenience. (Scott et al., 1991; Arni and Ward, 1996).

Both nontoxic mammalian pancreatic PLA_2 enzymes and highly toxic, pharmacologically active snake venom enzymes share common catalytic properties and a high degree of homology in primary, secondary and tertiary structures (Verheij, Slotboom and De Haas, 1981; Dufton and Hider, 1983; Renetseder et al., 1985). The precise structural features determining any of the pharmacological properties of venom enzymes have not been identified. These include presynaptic and /or postsynaptic neurotoxicity, myotoxicity, cardiotoxicity, initiation and /or inhibition of platelet aggregation, and hemolytic, anticoagulant, convulsant, hypotensive and edema-inducing effects (Kini and Evans, 1989).

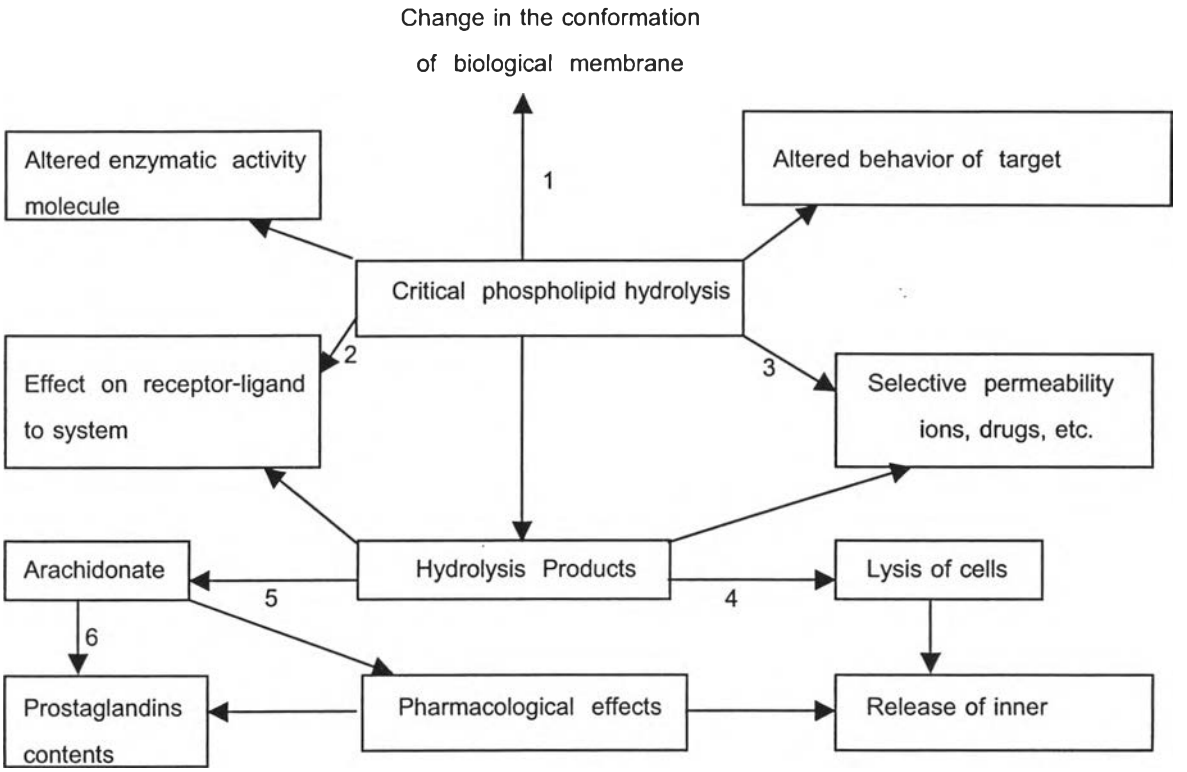

Figure 3. Mode and mechamism of action of PLA_2 enzyme, dependent on the enzymatic activity in the following way : (1) alteration in normal physiological function ; (2) interference of natural or synthetic lysophospholipids; (3) mobility of ions across the membrane; (4) liberated lysophospholipids may cause the lysis of cells (indirect hemolysis) ; (5) arachindonate released by venom PLA_2 (directly elicit the pharmacological effects) ; (6) released arachidonate may also cause increased levels of thromboxanes, prostaglandins and leukotrienes (Kini and Evans, 1989).

Table 3a. Phospholipase A_2 groups utilizing a catalytic histidine (Six and Dennis, 2000)

Groups	Initial/common source	Size (kDa)	Disulfides (number)	Unique disulfides	C-terminal extension	Chromosome Human	Mouse	Archetype enzyme	NCBI protein access
I	A : Cobra, Krait venom	13-15	7	11-77	None	N/A	N/A	Cobra	P15455
	B[b] : Mammal pancreases	13-15	7	11-77	None	12q23-24 [58]	5[33]	Human	NP_000919
II	A : Human synovial fluid , platelets, rattele snake, viper venom	13-15	7	50-137	7 res	1p34-36[26]	4[59]N/A	Human	NP_000291
	B[c] : Gaboon viper venom	13-15	6	50-137	6 res	N/A	4[26]	Viper	PSBGA
	C : Rat/mouse testis	15	8	50-137,86-92	7 res	1p34-36[26][d]	4[61]	Mouse	NP_032894
	D : Human/mouse pancreas/spleen	14-15	7	50-137	7 res	1p36,12[60]	4[33]	Human	NP_036532
	E : Human/mouse brain/heart/uterus	14-15	7	50-137	7 res	1p36[62]	4[33]	Human	AAF36541
	F[e] : Mouse testis/embryo	16-17	7	50-137	30 res	N,D	4[33]	Mouse	AAF04500
V	Mammal heart/lung/embryo	14	6	None	None	1p34-36[26]	4[26]	Human	NP_000920
X	Human spleen/thymus leukocyte	14	8	1-77,55-137	8 res	16p12-13,1[63]	16[33]	Human	NP_003552
III[f]	Bee/lizard/scorpion/human	15-18	5	N/A	N/A	22q[64]	N,D	Honey bee	P00630
IX	Snail venom (conodipine-M)	14	6	N/A	N/A	N/A	N/A	Marine snail	AAB33555
XI	A : Green rice schoots (PLA_2-I)	12,4	6	N/A	N/A	N/A	N/A	Rice	CAB40841
	B : (Green rice shoots (PLA_2-II)	12,9	6	N/A	N/A	N/A	N/A	Rice	CAB40842

[a] These are typical small extracellular PLA2's requiring millimolar [Ca^{2+}] and an active site histidine and aspartate pair. Note that Group V and X are listed after Group I and II because of their close homology with many conserved residues including 6+ disulfide bonds, and a histidine and two aspartates, as well as having N-terminal signal peptides that are cleaved to yield the mature PLA_2.

[b] Group IB has a five residue insert known as the pancreatic loop.

[c] Group IIB is missing one of the six highly conserved disulfides (approx. 61-94)

[d] group IIC is a psuedogene

[e] Human IIF has an additional Cys in its C-terminal extension.

[f] Human GIIIPLA$_2$ (55kDa) seems to possess additional novel C-terminal and N-terminal domains.

Table 3b : Phospholipase A_2 Groups utilizing a catalytic serine (Six and Dennis, 2000)

Group	Initial/common source	Alternate names emplyoed	Size (kDa)	Ca^{2+} effects	Characteristics	Human chromosome	Human NCBI access
IV	A : Human U937 cells/platelets RAW 264,7/rat kidney	cPLA2α	85	< μM : membrane transclocation	C2 domain, α/β-hydrolase regulatory phosphorylation	1q[65]	P47712
	B : Human pancrease/liver /heart/brain	cPLA2β	114	< μM : membrane transclocation	C2 domain, α/β-hydrolase	15[66]	AAD32135
	C : Human heart/skeletal muscle	cPLA2γ	61	None	Prenylated, α/β-hydrolase	19[66]	AAC32823
VI	A-1 : P388D1 macrophages, CHO	$iPLA_2$ or $iPLA_2$-A	84-85	None	Short splice, 8 ankyrin repeats	22q13,1[66,27,67]	AAD41722
	A-2 : Human-B lymphocytes, testis	$iPLA_2$-B	88-90	None	Long splice, 7 ankyrin repeats	22q13,1[66,27,67]	NP_003551
	B : Human heart/skeletal muscle	iPLA2γ or iPLA2-2	88	None	Membrane-bound	7q31[68,70]	BAA94997
VII	A : Human/ mouse/porcine /bovine plasma	PAF-AH	45	None	Secreted, α/β-hydrolase Ser/His/Asp triad in VIIA and B	N,D	Q13093
	B : Human/bovine live/kidney	PAF-AH(II)	40	None	Intracellular, myristoyated	N,D	Q99487
VII	A : Human brain	PAF-AHIb-α1 (subunit of trimer)	26	None	Intracellular, G protein fold Ser/His/Asp triad, dimeric	N,D	Q15102
	B : Human brain	PAF-AHIb-α2 (subunit of trimer)	26	None	Same as VIIIA :active as heterodimer or homodimer	11q23[69]	Q29459

[a] Larger, typically intracellular enzymes that utilize a nucleophilic serine for hydrolytic cleavage with no disulfide bonds and no Ca^2+ requirement for catalysis.

4.1.2.2.2.1. Pharmacological actions of phospholipase A_2

- ***Presynaptic neurotoxicity***

Presynaptic neurotoxic PLA_2 enzymes are the most potent toxins isolated from snake venoms. The neurotoxic site has also been identified mainly as the region between residues 59 and 92 (Dufton and Hider, 1983). This region overlaps with the anticoagulant region and comprises the β-sheet and its vicinities. Nerve terminals exposed to presynaptically active PLA_2 enzymes show mitochondria damage, depletion of synaptic vesicle and the presence of Ω indentations as the most common structural changes, and these are attributed to inhibition of vesicle recycling (Gopalakrishnakone and Hawgood, 1984; Cull-Candy et al., 1976; Landon et al., 1980). This has been interpreted as clear evidence for nerve terminal degradation (Harris, 1985). Thus, in presynaptic transmission blockade there are two functionally separate steps.

a) binding of PLA_2 enzymes to the specific presynaptic site which is unrelated to the enzymatic activity

b) pertubation of the presynaptic membrane by PLA_2 action near the binding site.

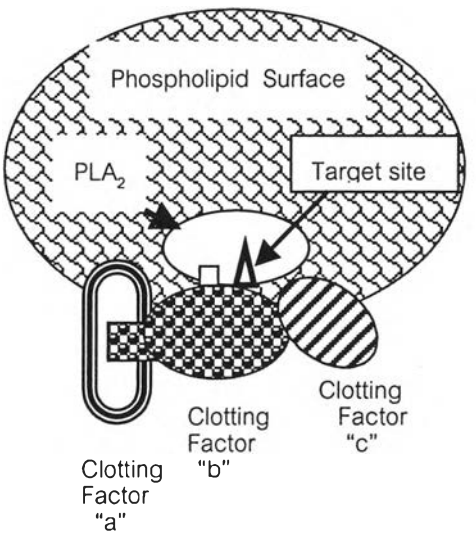

Figure 4. Model for the anticoagulant effect of PLA_2 enzyme. After binding to target clotting factor, the PLA_2 induces its anticoagulant effect by interfering with interaction of factors in the coagulation complex, or by hydrolyzing the phospholipid surface which is a critical component of the complex (Kini and Evans, 1989).

- *Anticoagulant activity* (Figure 4)

I) Thrombin effects

The prothrombinase complex is composed of factor Va, Xa, phospholipid and calcium ions. Snake venom phospholipases appear to inhibit formation of the prothrombinase complex by degrading phospholipids involved in this complex. The anticoagulant action results from the formation of a hydrolytic complex between the phospholipase and phosphatidylserine on platelet surface (Boffa and Boffa, 1976). Based on the potency of their action the phospholipases have been classified as strong, weak or non anticoagulant. The strong anticoagulants act to inhibit both the extrinsic factor X and the prothrombin activation complexes. The weak anticoagulants, by comparision, only inhibit the extrinsic factor X activation complex (Subburaju and Kini, 1997).

II)Platelet effects

Venom PLA_2 enzymes which interfere in platelet function can be classified into two distinct groups (Kini and Evans, 1989; Teng, Chen and Ouyang, 1984). Class A of platelet affector PLA_2 enzymes show biphasic effects on platelet aggregation (Ouyang and Huang, 1984; Teng, Chen and Ouyang, 1984). At low concentrations and with short incubation periods, these enzymes induce platelet aggregation, whereas high concentrations and with longer preincubation they inhibit platelet aggregation. Both the

initiation and inhibition of platelet aggregation by these enzymes have been related to the liberation of arachidonic acid by enzymatic activity (Ouyang and Huang, 1984; Teng, Chen and Ouyang, 1984). Class B PLA_2 enzymes inhibit, but do not initiate platelet aggregation. The antiplatelet activity of these PLA_2 enzymes seems to be independent of enzymatic activity (Huang, Yeh and Ouyang, 1984; Li et al., 1985). The mode of action for enzymes of class B is still unclear. The distinctly different pharmacological effects of these two groups of PLA_2 enzymes suggest the presence of distinct target molecules on the platelet surface and separate pharmacological site on the enzymes.

- Myotoxicity.

The phospholipase A_2 myotoxins lyse the plasma membrane of the affected muscle cell. They are found in the venoms of Crotaidae and Viperidae snakes and can be divided into 'Asp49' and 'Lys49' isoforms, the latter being considered catalytically-inactive variants (Maraganore et al., 1984). The presence of an aspartic acid at position 49 is crucial for calcium binding and Ca^{2+} is essential for catalytic activity (Scott et al., 1992). The lysine residue at position 49 has very low , or no hydrolytic activity. Despite the lack of an aspartic acid residue at position 49, the lysine PLA_2 proteins are very active in the induction of myonecrosis, by one or more still unknown mechanisms (Homsi-Brandeburgo et al., 1988; Johnson and Ownby, 1994). Whereas it is clear that the lysine PLA_2 myotoxins lyse the plasma membrane. The early

change, the plasma membrane , appearance of 'delta lesion' (wedge-shaped clear areas within the muscle cell lacking organelles), and cells with hypercontracted clumped myofibrils occur within the first 5-30 min. At the later time periods, these dense, hypercontracted clumps of myofibrils have become less dense and the cells have the more amorphous and hyaline appearance of necrotic cells (Ownby et al., 1999).

- *Cardiotoxicity*

Only a few PLA_2 enzymes from snake venoms show cardiotoxicity (Lee, Ho and Eaker, 1977; Fletcher et al., 1981, 1982; Chang, Lee and Lo, 1983). On the cardiac and neuromuscular muscles, the effects of the Asp-49 PLA_2 were accompanied by hydrolysis of phosphatidylcholine and phosphatidylethanolamine, whereas no phospholipid hydrolysis was observed with the Lys-49 PLA_2. Although the Lys-49 PLA_2 has much lower enzymatic and anticoagulant activities than the Asp-49 enzymes, but they show equal cardiotoxic and junctional effects. This suggests that PLA_2 enzymes exert effects independent of phospholipid hydrolysis (Dhillon et al., 1987).

5. Antivenom treatment and problems

Antivenom is prepared by immunizing horses with snake venom followed by fractionation of antibody from the immune serum. Inoculation of the crude venom provides the highest titre, however venom is often badly tolerated by the animal. As a result , toxoids have been prepared by biological detoxification of the venom which

preserve its immunogenicity. The venom preparation used for immunization is often associated with an adjuvant. The most commonly used adjuvants are Freund's, bentonite, aluminium hydroxide and sodium alginate. The immunization protocol depends on the toxicity and the immunogenicity of the venom, the animal target used for the immunization and the quality of the immune response of the animal (Chippaux annd Goyffon, 1991b). The preferred animal for immunization is the horse because of the large blood volume available, but other species can also be used. When a suitable antibody titre is reached, the blood of the animal is collected into an appropriate anticoagulant (e.g. sodium citrate). The immunoglobulins are digested using either pepsin to produce $F(ab')_2$ or papain to produce a smaller Fab fragment. Before final packing, the process of standardization of antivenom necessitates checking their neutralizing activities, their specificity, the stability and then ampuled and lyophilized preparations are made. Shelf times are 3 and 5 years for the liquid and lyophilized antivenoms, respectively. There are monospecific if only one venom is used in the immunization, or polyspecific if the immune animal receives a pool of venoms from several different species. The choice of either type depends on a range of considerations. In principle, a monospecific antivenom is more efficient for treating envenomation by the corresponding species. The specific treatment of envenomation is the administration of antivenom, i.e., the injection of empirical amounts of antibodies usually intravenously.

Although immunotherapy has proved its effectiveness in reducing mortality and morbidity of snake bites, it is also responsible for acute or delayed allergic reactions, the incidence depends mainly on the amount of heterogous antibodies injected and on

the purity of the antivenom. The dose is based on the identification of the snake, the time between bite and antivenom treatment, the clinical evolution of symptoms, the antivenom titre and the medical facilities available. As the precise amount of antivenom required for treatment is often unknown, it may be logical to administer antibodies in excess to eliminate all free toxins. However, the expected increased therapeutic benefit appears to be negligible (Riviere et al., 1997). And giving more horse serum protein than necessary is also dangerous (Cardoso et al., 1993). The dose of antivenom used depends on the resolution of the systemic signs of envenomation (e.g. restoration of blood coagulation, disapperance of haemorrhage, reversal of neurotoxic signs).

5.1. Problems of antivenom treatment

5.1.1. Hypersensitivity

Acute side effects are mainly caused by immune sensitization to horse antibodies, which are widely used in human therapeutics. Adverse reactions are due to the administration of foreign proteins after prior sensitization of the patient to horse serum, or to the presence of immune complexes. The first are non-antivenom specific reactions of type I hypersensitivity (HS) and are proportional to the purity of injected proteins. They appear a few minutes after the administration of antivenom. These reactions are usually benign (especially reactions of type I HS), but they can be life-threatening (anaphylxis).

- Early reactions appear either in sensitised subjects, having received previously therapeutic horse serum (antitetanus, antirabies, or antivenom), or in subjects who never received any therapeutic serum before. This reaction is

called anaphylactic (David, 1988). The presence of a high proportion of fragment Fc, lacking antibody activity but activating the complement, can induce an anaphylactoid shock (Paugh and Theakston, 1987). The prevalence of such accidents is variable (Ebisawa, 1973; Sutherland and Lovering, 1979; Sawai, 1980; Lagraulet and Pays, 1984; Malasit et al., 1986). Severe anaphylactic shock is rare, less than one in a thousand treatments (Chippaux and Goyffon, 1991a).

- Delayed reactions. Serum-sickness reactions are less dangerous but are more frequently reported. As a result, heterologous antibodies inducing the body to produce its own antibodies against the antivenom. Immune complexes settle on the intima of the small vessels and provoke a range of symptoms including fever , urticaria , adenopathy, arthralgia, nephropathy with proteinuria.

To improve the safety and efficacy of antivenoms, two approaches can be followed

a) improving the antivenom preparation and processing

b) opitimizing the use of antivenom

The improvement of antivenom therapy, it would be useful to optimize in the early stages of antivenom preparation. At present, there are few protocols to guide clinicians in the choice of an appropriate treatment of envenoming. To assess the necessity of immunotherapy and to modify the dose of antivenom, the severity of the envenoming must be known as quickly as possible. Moreover, some parameters of

antivenom administration, such as delay after snake bites, the route of administration and the type of antibodies to be used (immunoglobulins or $F(ab)'_2$) should be based on a clear understanding of the process of venom neutralization in the body.

5.1.2. Limitation of effectiveness of antivenom

Antivenom is effective in preventing death from respiratory arrest and CNS bleeding. It may not prevent local tissue necrosis at the site of venom injection consisting of hemorrhage, myonecrosis and edema (Ohsaka, 1979). In severe cases of envenoming, these local effects may lead to permanent tissue loss, disability and amputation. Clinical studies describe a generally limited effectiveness of antivenom serotherapy in preventing the development of local tissue damage (Reid, Thean and Martin, 1963; Warrell et al., 1976). This is due to several factors:

i) the lack (or scarcity) of antibodies to particular toxins involved in local tissue damage (Ownby et al., 1979; 1983)

ii) the fast action of these toxins, in comparision to the slow in vivo distribution of neutralizing antibodies, despite their presence in the circulation (Homma and Tu, 1970; Russell, Ruzic and Gonzalez, 1973; Ownby et al., 1984, 1986). The usually delayed administration of antivenom and the well established local tissue injury at the time of arrival at a medical center.

Since antivenoms have a limited efficacy in preventing local tissue necrosis, enzyme inhibitors could be of interest therapeutically, especially for preventing tissue necrosis caused by enzymes in snake venoms.

5.2. Contributing Factors to the severity of tissue necrosis

In many tropical countries with poor transportation facilities, non-availability of antivenoms, misdiagnosis and poor paramedical staff in rural clinics are contributing factors to the severity of local tissue necrosis.

5.2.1. Misdiagnosis.

Misdiagnosis of snake bite cases continues to be a serious problem in Thailand, ever though specific antivenoms are available. An incorrect diagnosis may derive from a bias toward the snake of high prevalence in a particular region. This is illustrated by the case of a man with severe systemic envenoming by a *C. rhodostoma*, who was misdiagnosed and mistreated as a Russell's viper bite. Lack of response to high dose of Russell's viper antivenom led to reevaluation of the diagnosis. Thus, 88 hours after the bite, *C. rhodostoma* antivenom was given, still producing a very dramatic response (Looareesuwan et al., 1988). Review of antivenom treatments , may help reveal the local level of misdiagnosis. Specifically, if many patients seem to need high doses of antivenom, the diagnoses may be wrong, and doctors should consider changing their bias toward another snakebite prevalence in that area.

5.2.2. Delayed treatment.

Delayed or lack of treatment with monospecific antivenom, especially in viperidae bites, may increase the chance and degree of local necrosis and chronic disability. Most such circumstances occurred as a result of patient's believes in traditional herbal treatment and when they went to hospitals only when their condition

deteriorated. For example, two patients who died of tetanus had been treated by herbal practitioners. Non–sterile plant material had been applied to their necrotic wounds. They arrived in a hospital 2 weeks later after developing rigidity and spasms (Looareesuwan, Viravan and Warrell, 1988).

5.2.3. Inadequate antivenoms.

The cost of antivenin can be as high as 20,000 bath per one case. Most persons bitten by snakes are poor and usually cannot afford the cost of treatment, resulting in none or inadequate antivenoms administered.

5.2.4. Tourniquets application

Patients bitten by venomous snakes frequently apply tourniquet to the affected limbs in an attempt to delay venom absorption into the circulation until antivenom is administered. However, the efficacy of this first-aid measure to reduce venom absorption and the severity of envenoming, is controversial. It has been shown that properly applied tourniquets delayed the onset of symptoms and the spread of the major toxin present in the venom of cobras until arrival at hospital (Watt et al., 1988). However, clinical studies in Burma and Thailand showed that tourniquets applied by patients in the paddy fields failed to inhibit the spread of venom into the general circulation (Ho et al., 1986; Tun et al., 1987; Khin et al., 1984). At present, tourniquet use has been discouraged in patients bitten by snakes whose venoms are associated with local necrotizing properties due to a high incidence of limb gangrene and other severe systemic complications (Pugh and Theakston, 1987). In the series of patients studied by

Amaral et al (1998), tourniquet application did not influence the severity of *Crotalus durissus* envenoming assessed by means of clinical and laboratory features and by the occurrence of complications. The frequency of local paresthesis, mylagia, palpebral ptosis, acute renal failure and respiratory failure, the median of plasma partial thromboplastine time and serum creatine kinase enzyme activity and number of deaths did not differ between patients who applied and did not apply tourniquets. They appeared to be ineffective in delaying venom absorption into the circulation. In view of the potential complications of tourniquets, which include gangrene, peripheral neuropathy and increased fibrinolytic activity, their use should be discouraged (Ho et al., 1986). It was also concluded that tourniquets are ineffective and should not be used in the management of viper bite (Nishioka, 2000).

5.3. Consequences of severity of tissue necrosis

In addition to systemic life-threatening effects, venoms from many species of snakes cause tissue damage at the bite site. This is classically defined as hemorrhage, myonecrosis, edema, fibrinogenolytic, fibrinolytic and kinin releasing activities, especially by snakes of the Crotalidae, Viperidae and some Elapidae families (Ohsaka, 1979).

In elapidae snake bite, necrosis of skin and subcutaneous tissue was generally evident by the end of the 1st week. About 58% of the necrotic lesions were complicated by pyrogenic infection. The skin loss varied from a few square centrimeters to almost the entire surface of the involved extremity. It was usually confined to the skin and subcutaneous tissue. When there was an extensive area of sloughed tissue, the time

required for healing was usually 1 - 2 month. The severity of neuromuscular signs correlates with the extent of the necrotic lesion , perhaps reflecting the amount of venom injected (Mitrakul et al., 1984).

Local toxicity of viper venoms frequently includes pain, swelling, echymoses which appear within minutes of the bite. Such signs are followed by necrosis of the area surrounding the bite site. Tissue necrosis often appears to be mainly ischaemic, developing slowly within a few weeks and presenting like dry gangrene. (Reid, and Theakston., 1983).

5.3.1. Skin graft and amputation

Necrosis of skin and subcutaneous tissue was frequently so extensive that skin grafting was required, and in some cases a certain degree of disfigurement persisted. Skin grafting involves the transfer of skin from healthy part of the body (donor site) to cover the injured area. The graft is said to "take" when new blood vessels and scar tissue form in the injured area. While most grafts from a person's own skin are successful, sometimes the graft doesn't take. In addition, all grafts leave some scarring at the donor and recipient sites. In severe cases of envenoming, these local effects may lead to permanent tissue loss, contractures, disability or amputation. Amputation of some part of the body causes impairment which leads to disability and ultimately to handicap. Handicap is defined a "measure of the disadvantage for a given individual, resulting from an impairment or disability that limits or prevents the fulfillment of a role that is normal for that individual". One consequence after snakebite is a series of restrictions of normal activities (e.g., shopping, visiting friends, engaging in sports and

hobbies). These operate as pivotal factors in health status indicators (e.g., pain and severity of illness symptoms) and depressive symptomatology (Williamson and Schulz, 1992a, 1995a). Pain and other illness symptoms contribute to depression to the extent that they restrict normal activities.

These phenomena have added to the injury due to the limited effectiveness of antivenom serotherapy in preventing the development of local tissue necrosis (Nishioka and Silveira, 1992; Cardoso et al., 1993). Thus, it is necessary to look for a new approach for the prevention and/or treatment of local tissue damage caused by snake envenomation. Since various hydrolytic enzymes, eg. phospholipase A_2 and metalloproteinase are known to be responsible for most of the local manifestations, it is interesting to study the effects of inhibitors of these enzymes in preventing or decreasing the local tissue damage. If effective, such a cocktail of enzyme inhibitors can be useful as a first-aid treatment (by local injection at the site of the bite) of snake envenomation.