CHAPTER 2

REVIEW OF LITERATURE
SECTION - 1

HISTORY, DISTRIBUTION & MORPHOLOGY
Since the Silurian and Carboniferous era the peoples of the Orient and of the Mediterranean region have been aware of the terror and strangeness of scorpions. The early Chaldean astronomers (Ca. 3000 BC) divided the Zodiac into twelve sections, one of which was called "Scorpio" with a scorpion as its symbol. The ancient Hindu astronomers also used the scorpion (śūrpa) as a subdivision of the Zodiac. The indications of scorpion had been found in Rig-Veda, hymn 191 (Circa 2000-900 BC). Ancient Egypt was also acquainted with scorpions, which was represented by the representation of the scorpion on ancient Egyptian monuments, the Ebers Papyrus and the Book of the Dead. Greek mythology also mentioned scorpions. Artemis, produced a scorpion which stung and killed Orion, son of Zeus, who turned Orion into the constellation that brought rain and storms. Scorpions had been mentioned as repugnant and formidable in the Bible and in the Talmud.

Moses Maimonides, in the twelfth century, mentioned certain stones to which folklore attributed therapeutic value for treatment of scorpion stings (Circa 1180). Ibn Khaldun, an Arab historian of the 14th century recounted the dangerous sting of scorpion.
In the religious art of the fourteenth, fifteenth and sixteenth centuries, the scorpion was often used as an emblem of the Jewish people to symbolise perfidy (Bulard, 1935). A painting by Fra Angelico, in the museum of San Marco in Florence, showed Christ carrying the cross, surrounded by three Roman soldiers wearing tunics ornamented with scorpions. The first investigation of scorpions, founded rather on experimentation and observations than on myth was performed by one of the first followers of the method of Descartes, Francesco Redi in 1668, who concluded that scorpions of Tunisia were often lethal.
Scorpions belong to the relatively small order of sub-class Arachnoidae, class Arachnida. The adult may vary in size from 2 to 25 cm. Even though 650 species of scorpions are known to exist, only a limited number (unrelated to size) are considered to be dangerous to man.

The scorpion has eight legs, two powerful claws, pinchers on pedipalps at the front of cephalothorax. There is another pair of small mouth pinchers (the chelicerae or mandibles) and on the underside of this, there are Pectines forming a pair of Comblike structure. The main body is divided into the cephalothorax followed by large preaddoms of seven segments and the tail (post-abdomen) with cylindrical bodies. The last segment of the tail, a bulbous enlargement, is called the telson; this bears coupled poison glands and leads to a curved sharp tipped terminal stinger.

The scorpion is a nocturnal animal, feeding on spiders and larger insects which it seizes in its claws, and stings to death with the tail coming over its head. In daytime it stays frequently hiding under debris, stones, in house corners, clothing and shoes. They live in marshy places in a colony under a depth of about 48 inches or more in the soil which is evident during their collection in rural areas.
GEOGRAPHICAL DISTRIBUTION

Only those species of scorpions which are considered to be dangerous to man are mentioned below:

a) Old world scorpions:

- **Heterometrus**, in India; **Pandinus**, in Arabia and Africa; **Opistophthalmus**, in South Africa; **Scorpio**, in North Africa; **Hadrurus**, in Malagasy and South Africa; **Androctonus**, from India and Persia to Atlantic coast of Morocco and Eastern Mediterranean region to Senegal and upper Egypt; **Buthsus**, from the Atlantic coast to Palestine, Senegal and Tunis; **Leiurus**, in Syria, Palestine, Egypt and Yemen; **Buthus**, in North Africa, Egypt, Ethiopia, Somalia, Palestine, Spain and France; **Parabuthus**, in the range from South Africa to Sudan; **Buthotus**, in Palestine, Syria, Algeria and Morocco.

b) South American scorpions:

**Tityus** are found in Mexico, the West Indies to South America, Argentina, Paraguay, Bolivia, Brazil, Peru, Ecuador, Colombia, the Guianas, Venezuela, Panama, Antilles, Puerto Rico, Trinidad, St. Lucia, St. Vincent and Grenada.

**Tityus serrulatus** & **Tityus bahiensis** are found mostly in Brazil. **Tityus trinitatus** is found in Trinidad and Venezuela.
c) Cantharidae are found in Arizona and some parts of California in USA Central and South America.

d) Euscorpions are found in Asia Minor, European Mediterranean region, Caucasus and North Africa.

e) Hadurus are found in the Southern States of USA and Mexico.

f) Vejovis occur over a wide region of North America.
SECTION - 2

COLLECTION OF SCORPION VENOM
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The venom of the scorpion (Heterometrus bengalensis) may be obtained by applying several methods.

Jousset de Belleme (1872) obtained Scorpion Venom by maceration of the crushed telson in distilled water/physiological saline. In this method, the material was thereafter dried over CaCl₂, ground, dissolved in 0.9% NaCl and periodically agitated in the cold (2° - 4°) and centrifuged. The solution contained soluble tissue products in addition to the venom. This method was convenient for the preparation of toxin used in the production of immune serum. However, as substances other than the venom were present in this preparation, the problem remained, as to whether a given toxic response was due to the venom.

Venom collection by electrical stimulation of the telson was a better method. This contracted venom gland muscularis; venom droplets appeared at the orifice of the stinger and were easily collected. The first few drops were more toxic than the following drops (Físalix and Varigly, 1896). Físalix (1922) suggested that the venomous granules secreted by the glandular cells probably reacted chemically with substances in the glandular liquid to produce the final venom.

The fresh natural venom had a neutral-to-alkaline reaction while...
Immature venom was acid. Both had a foamy whitish appearance, with stringent properties and without any taste on the tongue. The vacuum-dried venom was whitish gray, hygroscopic and might be stored in a vacuum desiccator, over fused calcium chloride at room temperature and in the dark without apparent change of activity for several months (Bücherl, 1971), when dried venom powder was refrigerated in dry ampoules it remained potent for many years (Baloset, 1971).

The quantity of venom obtained depended on the species, state of captivity and extent of repetition of electric extraction (Baloset, 1971; Bücherl, 1971).

Zlotkin and Shulov (1969) employed mechanical stimuli to make the scorpion sting a parafilm sheet. Manual compression of telson with forceps had also been used to extract venom from certain species of scorpions (Hedogone and Ophiopthalmus) in which the chitin of the telson were sufficiently supple (Kranz, 1970; Grasset, Schanssen and Hadson, 1946).

It was difficult to compare the yields of toxins obtained by telson maceration and electrical telson stimulation. The toxic substance of the telsons were present in the venom-producing glands in the form of liquid protids and as granules in the cytoplasm of the epithelial cells; the total amount of toxicity was greater than that obtained by electrical stimulation (Baloset, 1956). The residual toxicity of the venom apparatus after the extraction of the venom was approximately one-fourth of the total toxicity of gland plus venom. This marked
difference probably arose from a reduction in the toxicity of venom obtained by this method (Lissitsky, Miranda, Etzenberger and Mercier, 1956).
SECTION - 3

PHYSICOCHEMICAL PROPERTIES AND FRACTIONATION
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Like other animal toxins, scorpion venom was soluble in distilled water (Balozet, 1971), physiological saline solution or in glycerine. The insoluble part had the consistency of a mucous, could be separated by centrifugation and lost all toxicity after washing with distilled water or in physiological saline. The soluble component of the dried venom contained the toxins. The active part was generally insoluble in neutral solvents i.e., ethyl and methyl alcohol, ether, chloroform, acetone, benzene, xylene, or oils. It was not retained by Chamberland or Berkefeld filters of medium porosity and was only partially dialyzable (Balozet, 1971).

Scorpion venom was found to be thermostable for many species. These were Centruroides sculpturatus and Leiurus quinquestriatus (Flurry, 1923; Megalhaes, 1965; Watt, 1966; Balozet, 1971; Nitzan and Shulov, 1971; Nitzan, 1970).

Scorpion venom was antigenic and composed of several protein fractions which had been separated by various methods, such as electrophoresis, ion-exchange chromatography, sephadex G gel filtration (Russel, Karlson, Smiffer and Biggare, 1970 and Zlotkin, 1970), solvent extraction, thin layer chromatography and adsorption chromatography. Not all of the separated fractions were toxic and their actions differed (Zlotkin, 1970). The composition of the venom might vary according to the collection procedure, nutritional status, age, species and season.
Protein had generally been reported as the main constituent of scorpion venom (Wilson, 1904, 1921; Mohamod, 1944; Miranda and Lissitzky, 1961; Watt, 1964; Stahnke, 1965; Xarom, 1970; Zlotkin, Miranda, Kupyan and Lissitzky, 1971, Dinis, 1971; Baloset, 1971; Nitzan, 1971; Zlotkin, Miranda and Lissitzky, 1972). The telson extract of Egyptian scorpion venom contained two proteins which coagulated at 56° and 76° (Wilson, 1904, 1921).

The chemical nature of the Buthus Sp. venom was found to be a proteose (Mohamod, 1944). Miranda and Lissitzky (1961) found that Androctonus australis and Buthus occitanus venom contained two toxic proteins, which these workers designated as acorpamine.

Centruroides sculpturatus venom was reported to contain proteins and peptides but apparently did not contain polysaccharides (Watt, 1964); Stahnke (1965) on the other hand reported the presence of polysaccharides.

On electrophoretic separation, Centruroides venom (Boliver and Ornelas, 1949; Boliver and Rodriguez, 1955) and Tityus (Dinis and Goncalves, 1956; Fisher and Bohm, 1957) and Buthus judaicus venom (Weisman, Shulov, and Schafier, 1958) yielded 8 and 6-7 protein fractions respectively. In Androctonus australis one or two fractions had been detected (Baloset, 1960). Miranda and Lissitzky (1958), and Miranda, Rochat and Lissitzky (1960, 1963 and 1964 a, b, c)
obtained homogenous neurotoxins of the venoms or telson extract of *Androctonus australis* and *Buthua occitanua* by employing ion-exchange chromatography and gel filtration and found two toxins ("scorpanes") which seemed to be basic proteins of low molecular weight (10,000 - 18,000) with a high proportion of cystine and basic amino acid in conformation (Miranda, 1964). *Johnson, Toller and Stahnke (1966) showed that in venoms of 18 different species of scorpions as well as purified toxins there was no correlation between lethality to mice and paralysis of insect larvae (Zlotkin, Miranda, Kupyan and Lisitsky, 1971, 1972) and the toxin protein (the "insect toxin") was purified from the venom of the scorpion, *A. australis*. The final purified product was a single chained protein of low molecular weight. It was interesting to note that the presence of a disulfide bridge in position 12 was common to all scorpion toxins active on mammals, whereas it was occupied by lysine in the insect toxin. On the basis of currently available data further surmise concerning structure function relationships of the insect toxin would not be prudent. Discrete larval-toxic proteins had been demonstrated in six other scorpion venoms (Zlotkin, Miranda and Lisitsky, 1972).

An additional toxin selective for crustaceans was isolated and purified from *A. australis* scorpion (Zlotkin, Miranda, and Lisitsky, 1972; Zlotkin, Miranda and Lisitsky, Unpublished). The insect toxin was able to block the induced afferent transynaptic response at the sixth abdominal ganglion of the cockroach (P’Ajello, 19...
Zlotkin, Miranda, Lissitzky and Bettini, 1972). The crustacean toxin, in contrast to insect and mammal toxins, was able to block the crayfish stretch receptor organ (Pansa; Miglioli-Natalli and Bettini, 1973).

McIntosh and Watt (1972) isolated and characterized four different polypeptide toxins from the venom of North American Scorpion, Centruroides sculpturatus. These toxins were toxic in varying degree to vertebrates and invertebrates.

Scorpion toxins were generally difficult to separate due to their similarity in size and charge. Miranda (1962) employed a reversible retention method of Sephadex to isolate two toxic fractions of F. gravimanus venom. These toxins were polypeptides of small molecular weight (1000). Their properties were very similar to those of the North African scorpions (Miranda, 1970) and North American Scorpions (McIntosh and Watt, 1972). Partial study of amino terminal sequence of the toxic protein of C. sculpturatus showed similarity with the insect and mice toxins from the North African Scorpion, J. australis (Watt, 1974).

El-Ammar, Osman and Ismail (1973) also fractionated Buthus nigra venom by electrophoresis into seven components; two were lethal to mice. One of the lethal fractions increased but the other decreased the amplitude of the twitches of the rat phrenic-nerve diaphragm preparation.

El-Asmar, Ibrahim & Zabil (1972) had been able to fractionate the venom of *Leirus quinquestriatus* into nine components by a combination of electrophoresis and column chromatography, three of which were highly toxic, the other two fractions caused hemorrhages.

Electrically-stimulated *Tityus serrulatus*, *Tityus bahiensis* venom contained proteins and several protein fractions (Diniz, 1971). Diniz (1971) separated the venom by a combination of solvent extraction, chromatography and polyacrylamide gel electrophoresis into three biologically active fractions. The separated fractions were toxic, smooth muscle contracting, capillary permeability increasing and had some hyaluronidase activity. "*Tityus toxin*" was similarly purified from *T. serrulatus* venom and was 16 to 20 times more toxic than the whole venom. The homogeneity of "*Tityus toxin*" was well-documented by the electrophoretic criteria, by Polyacrylamide gel, and by chromatographic procedures.

Babu, Bass and Venkatachari (1971) separated by electrophoresis the venom of *Heterometrus fulvescens* into six protein fractions at pH 8.6 and all of which were exhibited Cathodic mobility.
Lelurus quinqueatratus venom was studied by Nitzan and Shulov (1971) for its toxicity. Protein content and electrophoretic nature of its protein contents might undergo qualitative changes after lyophilization.

Enzymes: Master, Rao and Soman (1963) separated Natrix tamaluus, Palamneus gravlmanus venom by stretch gel electrophoresis and studied the biological activity in different bands. Protease was present in both venoms, 5-nucleotidase in the latter and phosphodiesterase in the former. In contrast to snake venom there was no phospholipase A, cholinesterase, or L-amino acid oxidase in these species.

Kurup (1965) estimated the phospholipase A activity in Heterometrus scaber venom but proteolytic activity was absent. Human serum albumin and reducing agents (but not oxidizing agents or globulin) inhibited the phospholipase A activity. Reducing agents like cystine and glutathione might possibly had broken the disulfide bridge of the enzyme molecule necessary for its action.

Direct correlation between enzyme activity and toxicity were not shown, but the enzyme activity was found by Kurup (1965) to be located in a low-toxicity fraction of the venoms obtained by ammonium sulphate precipitation. This fraction could be further subdivided into two fractions one of which showed phospholipase A activity. Highly toxic fraction of the venom, obtained by 0.9 saturation, had very little enzyme activity. The enzyme required co-factor for its activity. Calcium acted as such a co-factor. The partially thermostable enzyme activity
could be inhibited by certain metals, glutathione, cystine, EDTA, and citrate.

The presence of phospholipase A in the venom of several North African Scorpions had been reported by Ibrahim (1967); Mohamed, Kamel and Ayobe (1969) found phospholipase A activity to be of low order. Enzyme activation could be enhanced upon addition of ether, sodium deoxycholate, calcium and magnesium ions, but inhibited with EDTA combined with sodium deoxycholate and calcium ion. The phospholipase B activity could be inhibited by EDTA and ether. The venom was found to contain heat-labile proteinase, cholinesterase, amylase, glutamic pyruvic transaminase and glutamic oxaloacetic transaminase.

Lecithinase activity in *Scorpio maurus* venom and hyaluronidase activity in *Buthus bicolor* (A. australis), *Eurypilus italica*um, *Buthus ocellatus* and *Scorpio maurus* had been studied by Balozet (1971). *Buthus ocellatus* and *Scorpio maurus* venom possessed hyaluronidase activity. Several mammalian sera inhibited the enzyme activity. There was no proteolytic activity in the venom of *Latrodectus quinquestriatus*, *Androctonus australis*, *A. anorexii*, *B. ocellatus*, *B. judaeus*, *S. maurus*.

Balt, Dass and Venkateshri (1971) reported inhibitor activity of *Heterometrus fuliceps* venom on cockroach muscle enzyme activity (succinic dehydrogenase, lactate dehydrogenase, acetylcholinesterase) were inhibited.

*Scorpio maurus palmatus* venom contained gelatinase and anticholinesterase.
Venom from this species as well as Bathus quinquemaculatus contained hyaluronidase which would help the spreading activity of venom (Moharoed, Kamel and Ayobe, 1973).

Heterometrus acaber venom contained RNase, 5-nucleotidase, hyaluronidase, acetylcholinesterase, acid phosphatase and phospholipase A (Hair and Kurup (1973). They showed strong hemolytic activity in presence of lecithin; and succinic dehydrogenase inhibitor activity (Hair and Kurup, 1973).

Protein sequencing and its activity:

A remarkable feature of the amino acid composition in scorpion venom was the high content of aromatic amino acids (5–14 per molecule), especially tyrosine (3–6). The amino acid sequences of the two toxins from Androctonus australis Hector were very similar to each other (Iochet, Rochet, Miranda, Lissitzky and Edman, 1970). Lissitzky and Edman (1970) showed that more than 20 residues of the N-terminal sequence had been determined for 7 other toxins.

The scorpion toxins were basic proteins, but the presence of an essential cationic group was not yet certain.

5-Hydroxytryptamine:

Linis and Gonclaves (1956) suggested the presence of 5-HT in the venom of Tityus on the basis of paper chromatography and fluorometry. Adam and Weiss (1956, 1958) reported the presence of 5-HT (2–4.5 μg/mg venom) in the venom of

*Heterometrus aenobar venom* (Hair, Raj and Kurup, 1973) had been shown to contain six indole compounds, four of which were identified as tryptophan and serotonin (3.8 mg/gm of dry venom). 5-HT in *Pseudorattus cristalis* was reported by Osman, Gumma and Karrar (1974).

**Histamine:**

*P. gravissimus* venom had been shown to contain histamine (Ismail, 1975).
SECTION 4

PHARMACODYNAMICS
Until the beginning of the eighteenth century there had been very few investigations of poisoning due to scorpion venom. de Maupertuis (1931) experimented on dogs and chickens. Mancary (1810) tried the stings of Buthus occitanus on himself. Bert (1865, 1885), Jouassat de Belleaus (1872, 1874), Valin (1876), Joyeux - Laffitte (1882, 1883) also contributed to the literature on scorpionism.

Until recently several workers demonstrated obvious changes in cardiovascular system such as hypertension sometimes followed or preceded by hypotension, inotropic, chronotropic electrocardiographic pattern, myocarditis and increase in capillary permeability.

Caius and Hbasker (1932) reported that with Buthus aseneus, Palamneua, Isometria and asometria venom blood pressure was increased. The rise in blood pressure occurred in decerebrated and spinal animals and was independent of vagus control. With Palamneua venom, at first there was a fall and thereafter a rise of approximately 15-20 minutes duration. These changes persisted in vagotomised and decerebrated animals. The rise in blood pressure was always smaller where compared with Buthus venom. Isometria venom had similar action like Palamneua venom but the changes were much less marked; no action of
Scorpions' venom could be demonstrated on heart or blood pressure. They reported cardiotonic action of *Buthus* venom. They suggested on the basis of these observations that the rise of blood pressure in anesthetized animals was due to action of venom on both cardiac musculature and on peripheral blood vessel and this effect was independent of the central nervous system.

Rao, Premlatha, Venkata Krishna Bhatt and Haranath (1969) showed that *Buthus tamulus* venom lowered dog blood pressure. With smaller doses there was a gradual decline of blood pressure after a slight initial rise.

*Buthus quinquestriatus* venom was shown to raise blood pressure (Mohamed, 1942, 1950), an action shared by *Buthus sinaloae* venom (Ismail, Osman and El-Aamar, 1973). They showed that this hypertensive response was blocked by alpha-adrenergic blockers. The hypertension in cats and rats was preceded by brief atropine-sensitive hypotension.

del Pozo (1956) showed that the venom of *Centruroides* sp., *Scorpio maurus* and *Tityus serrulatus* raised blood pressure in experimental animals. He suggested that the hypertensive response resulted from activation of vasoconstrictor spinal neurones as shown by absence of such response after destruction of the spinal cord and potentiation in decerebrated animals. According to him, adrenaline secretion through spinal stimulation was another factor in the hypertensive response. Corrado, Massimo Metro and Antonio (1974) studied the mechanism and site of
action of the hypertensive response to Brazilian scorpion (*Tityus serrulatus*) venom. Their results were consistent with an indirect action of the venom through the release of catecholamines.

Freire-Maia, Pinto and Franco (1974) suggested that the pressor effect of venom (*Tityus serrulatus*) was caused by the release of catecholamines from adrenal glands and post-ganglionic nerve endings, and the hypotensive effects, which occurred before or after pressor effect (with higher doses of toxin) were due at least partly to sinoatrial, sinoatrial and atrioventricular blockade.

*Centruroides sculpturatus* venom (Patterson, 1960) showed a typical hypertensive response in cat. The vasoconstriction was ascribed to pressor substances rather than to sympathetic activity, since sympathectomy did not prevent vasoconstriction; the adrenal gland was implicated as the source of pressor agent. They also suggested that the venom either had a direct vasoconstrictor effect or an effect mediated by central pressor centres.

Ismail, Osman, Ibrahim and El-Asmar (1972) also reported that the *Leirus quinquepustulatus* venom produced a short-lasting hypotension followed by a pronounced hypertensive response that lasted 10-15 min. Treatment with atropine blocked the initial hypotensive effect of the venom and potentiated the subsequent hypertension. The hypertensive effect was blocked by treatment with
Phenoxybenzamine. Phenoxybenzamine pretreatment clearly revealed the hypotensive action of the venom, which was blocked by atropine. Carotid sinus and body denervation and/or bilateral vagotomy before or after carotid sinus and body denervation markedly potentiated the hypertensive effect of the venom and prolonged its duration.

Caius and Mhasker (1932) showed that with Palamneua venom, at first there was a fall and thereafter a rise of approximately 15-20 minutes duration. These changes persisted in decerebrated or vagotomized animals. Isometrua venom had similar action like Palamneua venom but the changes were much less marked.

Buthus tamulus venom lowered dog blood pressure, with smaller doses there was a gradual decline after a slight initial rise (Nao, Premlatha, Bhatt and Haranath, 1969).

Crude Vejovia spinigerous venom administered intravenously had been described to lower blood pressure followed immediately by a hypertensive response (Nasse, 1968).

With Buthus toxum venom mortality was associated with E.C.G. changes suggestive of toxic myocarditis. With smaller amounts of venom, tachycardia was found (Nao, Premlatha, Bhatt and Haranath, 1969).

Buthus quinquestriatus venom also was reported to increase the rate of isolated heart preparations, the tachycardia being blocked by ergotoxin.
Mohamed (1942),

*Buthus minax* venom increased force of contraction but not rate of isolated guineapig and rabbit heart (Ismail, Osman and El-Asmar, 1973). In reserpinized and in normal hearts, the venom produced bradycardia which was blocked by atropine.

Cheymol, Beurellet and Rocab-Arvéillet and Hédi Aloui (1974) reported complex cardiovascular action of the venom of *Androctonus australis*, *Buthus occitanus* and *Leiurus quinquestriatus*. These reduced contraction and rate of heart; later rate was enhanced, and fibrillation might occurred. They suggested involvement of muscarinic intracardiac receptors and beta-adrenergic receptors. These produced a strongest discharge of catecholamines and the cardiotonic effect was hardly antagonized by propranolol.

Ismail, Osman, Gumma and Karrar (1974) reported that *Pandinus exitatus* venom increased amplitude of contraction in isolated rabbit heart though rate increased only slightly. After reserpine pretreatment, venom produced first a reduction, and then an increase of rate and force of contraction of heart.

They reported that the histological lesions resembled those described as a result of catecholamine overdose which they suggested the venom had a sympathomimetic action on the dog heart.

Caiua and Mhasker (1932) reported that in the heart of dog and rabbit
Buthus tamulug, Palamneus swammerdame and Palamneas fulvipes venom, caused a diminished coronary outflow suggesting coronary arteriolar constriction.

Venom from the last two species mentioned were less potent in this regard.
RESPIRATORY SYSTEM:

Scorpion venoms cause respiratory changes. Among all the Mexican scorpions examined, *Centruroides Sp.* was marked for respiratory paralysis which del Pozo (1956) suggested that it might be main cause of death.

Patterson (1960, 1964) studied *Centruroides sculpturatus* venom on cats, dogs and rats. He found an initial period of respiratory arrest which persisted for 20-30 seconds following administration of venom. All animals developed gasping which progressively diminished in amplitude and frequency until death resulted from anoxia.

Rosin (1969) reported the increased respiration and spasms in experimental mice with *Hobo hierochonticus* venom.

Ismail, Osman, Ibrahim and El-Aamar (1972) reported immediate decrease in rate and depth of respiration alternating with hyperpnoic periods with *Leiurus quinquestratus* venom. Repeated injection of venom the respiration became periodic. Intracisternal administration caused immediate respiratory paralysis had been reported by del Pozo (1956).

Freire-Maia, Ribeiro and Beraldo (1970) found apnoea in rats, an effect not abolished by vagotomy, with *Tityus serrulatus* venom.

They suggested that the toxin produced inhibition of respiratory centre caused stimulation of the expiratory centre. They observed "gaping" breathing
after toxin administration. However, they stated that respiratory arrhythmias might be produced by actions of the toxins on peripheral neuromuscular transmission (del Pono, 1966; Adam-Weiss, 1956; Torres and Diniz, 1964; Wittal Brazil, 1966; Reisel, 1967 and Diniz and Torres, 1968).

Tityus serrulatus toxins produced respiratory arrhythmias in rats (Freire-Maia, Azevedo and Costa Vel, 1973). Freire-Maia et al (1970) assumed that gasping and ataxic respiration were due to stimulation of the chemoreceptors in the carotid and aortic bodies, a suggestion supported by Patterson and Wolly (1970). They suggested that sensory receptors for the toxin could be in the lungs, apnoea being caused by a continuous firing of pulmonary receptors.

Heterometrus fulvipes venom caused increase consumption of oxygen which returned to normal in about five minutes in cockroach (Babu, Dass and Venkataschari, 1971). This gradually decreased to a very low value in about forty minutes.
Scorpion venom was a source of low molecular neurotoxic proteins (Miranda and Lissitsky, 1961; Miranda, Rochat and Lissitsky, 1964; Watt, 1964; Miranda, Rochat, Rochat and Lissitsky, 1966; Gomez and Dixis, 1966; Zlotkin, Blondheim and Shulov, 1970). It might be supposed that the depolarising and blocking effects of the crude scorpion venom on nerves (Koppenhöfer and Schmidt, 1965; Zlotkin, Blondheim and Shulov, 1970), muscles (Adam and Veys, 1959; Cheymol, Bojrillet, Roch-Arvellier and Heux, 1974) and the neuromuscular junctions (La Grange, 1974) in vertebrate systems were due to these toxins. Recently 15 toxins derived from several scorpion venoms (Miranda, Kupyan, Rochat, Rochat and Lissitsky, 1964; McIntosh and Watt, 1972) were isolated and purified. It was found that they were single-chained, mainly basic, polypeptides cross-linked by four disulfide bridges. At the level of the amino acid composition, these compounds had some features in common: low molecular weight (varying between 6,500 and 9,000 with the majority around 7,000); absence of methionine (a common feature of the majority of snake toxins as well); presence of 8 half-cystines (except in one toxin) which according to Miranda (1979) all were involved in disulfide bridging; low histidine and phenylalanine contents. Beyond these resemblances in their composition they appeared to be quite diverse and were much more heterogeneous than the elapid and hydridid snake toxins (Cu, 1963; Jimenez-Peñas, 1968). Considering the heterogeneity in composition of A. Androctonus
australis toxins it is was interesting to notice the relatively high level of homogeneity in their N-terminal primary structure.

Primary structures of some of the toxins showed greatest extent of homology at the N-terminal section of the molecule which gradually decreased towards the C-terminal end. It was suggested by Rochat, Roca, Kapuyan, Lissitzky, Miranda, Rabi and Edman (1970) that N-terminal sequence might play an important role in the biological functions. It was proposed that the amino acid in position 10 was of particular importance and the replacement of a neutral residue by a basic residue might be associated with a decrease of the specific toxicity (Rochat, Rochat, Kapuyan, Miranda, Lissitzky and Edman, 1970).

The structure of the toxins derived from the venom of the scorpion Androctonus australis Hector might serve as a typical example of taxonomical implication derived from chemical information. Upto now the subspecies Androctonus australis Hector was considered, from the taxonomical point of view, as being homogeneous (Vaeleon, 1952). However, the interchange between toxins A III and A I; which must be a genetic characteristic, suggested a taxonomical heterogeneity.

Some of the neurotoxins acted peripherally. Among all others, Tityus serrulatus and Tityus bahiensis were important in respect to their neurotoxic activity. Neurotoxic effects reported in mice with Helo hierochunticus (Rosin, 1969).
del Pozo (1962) reported the neurotoxic effects of the scorpion venom on the ventral roots of the centrally sectioned spinal cord and of the muscle innervated by it. He showed that the venom induced several potential spikes in response to stimuli applied to the middle part of the nerve. These discharges had their origin in the motor plate region when the nerve was sectioned near its muscular end, the repeated potential disappeared and the normal electrical responses persisted. This experiments showed that the increase in width and length of the muscular contractions observed with scorpion venom was due to an effect of the neuromuscular junction and he suggested that this phenomenon might occur in the motor plate.

The mechanism of depolarizing excitatory effects of the scorpion venom on an excitable membrane was investigated by several workers (Adam, Schmidt, Stampfl and Weiss, 1966; Adam and Weiss, 1966; Koppenshoffer and Schmidt, 1966). Scorpion venom was applied to the node of Ranvier in an isolated nerve of frog by perfusion. Adam, Schmidt, Stampfl and Weiss (1966) shown that there was prolonged duration of action potentials and spontaneous activity caused by the application of scorpion venom. They concluded that the main action expressed as an increase of the sodium permeability of the resting membrane and of delayed inactivation of this permeability.

Adam and Weiss (1969) reported that application of scorpion venom to denervated preparations of skeletal muscles caused a repetitive contracture of
skeletal muscle. Under the influence of the venom the resting membrane potential of a muscle demonstrated slow depolarisation until the threshold level of the action potential was reached. Veratrine, known to have direct depolarisation effect on skeletal muscles and nerve fibres possibly due to an increase in the influx of sodium ion, could imitate the above effects of the venom when applied to muscle preparations. It was suggested (Adam and Weiss, 1966) that the scorpion venom might have a similar mechanism of action. On the contrary, *Hymenometra serrulatus* venom was shown to affect the muscles only indirectly through a nerve by stimulating the release of a transmitter substance (Brazil, as in Adam & Weiss, 1966).

The action of many types of scorpion venom has been widely reported (del Pozo and Aquiano, 1947; Adam and Weiss, 1959; Zlotkin, Blondheim and Shenov as cited by Zlotkin, 1969; Parnas and Russel, 1967). Intact nerve trunks were relatively resistant to venom. Nerve blockage by venom was only achieved after prior desheathing pretreatment with pronase. This blockage was preceded by appearance of strong spontaneous action potentials, thus demonstrating the excitatory effects of the venom on an exposed nerve.

Ismail, Osman and El-Asmar (1973) reported that *Buthus asterias* venom increased the size of the twitches of the isolated, directly stimulated, rat phrenic-nerve diaphragm, suggestive of venom-induced slow depolarisation of the excitable nerve membrane and muscle which was possibly due to a delay in the inactivation of sodium.
Thus the occurrence of neuromuscular intoxication was due to the action of scorpion venom on exposed fibres and muscles, directly or through motor nerve. An intact nerve trunk would appear impermeable to scorpion venom, the venom acted on the nerve tissue after contact at the exposed presynaptic terminals. The resulting spontaneous muscular twitchings and fibrillation were probably due to an quantal release of transmitter substance stimulating the post synaptic membrane. Muscle contractions, action potential in nerve fibres and release of a transmitter substance at nerve terminals were all dependent on a common primary process; the depolarisation of excitable membranes (Katz, 1966). It would appear that scorpion venoms would able to perform this basic function.

Gorrado, Antonio and Diniz (1968) reported that the venom of Tityus serrulatus produced a very marked cardiac stimulation. The cardiac stimulation (produced by the venom) was blocked by the beta-sympatholytic agent and was absent in reserpine-treated heart. These authors suggested an indirect action of the venom probably through the release of tissue catecholamines. They also mentioned that the venom potentiated the hypertensive response of the dog to injected catecholamines, and to isometric conclusion possibly indicating its participation in the mechanism of inactivation of catecholamines. Hypertension attributed to a generalised sympathetic stimulation might be peripheral or central through the pressor centres and adrenal activating neurons (del Pozo, 1966; Patterson, 1960; Gorrado, Antonio and Diniz, 1968; Goñin, Stern and Cohen, 1967). Henriques, Cassinelli, Diniz and Gomes (1968) supported the latter.
possibility by maintaining that the venom produced adrenal gland catecholamine depletion which was blocked by adrenal gland denervation.

The effect of scorpion venom on the guineapig ileum was studied by Diniz and Goncalves (1956). They suggested that the venom caused an activation of the parasympathetic post-ganglionic nerves. This effect was inhibited by atropine increased by eserine and unaffected by hexamethonium. Diniz and Torres (1960) showed that fragments of guinea-pig ileum incubated with scorpion venom in presence of eserine released an acetylcholine-like substance. This was inhibited by morphine. To these workers, it appeared that the cholinergic influence of the venom was due not only to an activation of motor neurones but also to a general acceleration of acetylcholine release in the tissues.

Patterson (1960) reported salivation and mydriasis in experimental animals with Centruroides sculpturatus venom. He suggested an initial peripheral action followed by an action dependent on the central nervous system.

D’Alessio, Zlotkin, Miranda, Lissitzky and Bettini (1972) reported that the crude venom of Androctonus australis Hector and its “insect toxin fraction” (but not “mammal toxin”) blocked induced afferent transynaptic responses in cockroach. They suggested that the toxicity of the insect and mammal toxins of the venom was based on specific affinity of venom to the nervous systems of different species of animals.

del Pozo and co-workers (del Pozo and Anguiano, 1947; del Pozo and
Derbez, 1949; Del Pozo, 1964) suggested that the spontaneous muscular twitches and fibrillation seen after intravenous administration of Mexican scorpion venom were due to an effect on the motor neurons from the spinal cord or to a direct effect on the neuromuscular junction.

Parmas and Rusell (1967) showed that the venom of several North American Scorpions produced both blocking and excitatory effects, when electrical stimulation was applied to the nerve, the muscle response was blocked by the venom, but the muscle was able to respond to direct stimuli.

Diniz and Valeri (1969) reported that *Tityus serrulatus* venom produced intense salivation and lacrimation in mice which was not significantly affected by hexamethonium, d-tubocurarine or morphine.
Scorpion venom-induced twitches and contractures in skeletal muscle has been reported by a number of workers.

Del Pozo (1956) reported the appearance of spontaneous muscular twitches and fibrillation after injection of Centruroides sp., Scorpion venoms and Tibius serrulatus venom, which he ascribed to central action on spinal motor-neurones. Abolition of the brain and excision of spinal cord abolished this effect. Intra arterial injections of the venoms and local application to the muscles, also provoked twitches and fibrillation, the last effect being due to a peripheral action on the neuromuscular junction. Peripheral nerves or denervated muscles were not activated even with very high concentration of venom.

When stimulation (electric/aceylcholine) was applied to venom-treated muscle larger and longer contractions occurred. Responses to single shocks became repetitive; response to a series of shocks, resembled tetani resulting from stimulation at higher frequencies. On successive activation there was progressive decline in both repetition and amplitude of responses.

Patterson (1960) reported peripheral asynchronous skeletal muscle twitchings when Centruroides sculpturatus venom was injected into the dorsal lymph sac of frogs. There was no reduction in the muscle response in brain-pithed frogs, but some twitchings persisted in the double-pithed frogs.
After unilateral sciatic nerve transection, there was no twitching regions posterior to the level of transection. Since sciatic nerve transection prevented the response, the slight effect in the spinal pithed frogs was believed to have resulted from the incomplete destruction of spinal motor centres. Tubocurarine was found to suppress muscle twitching in both venom-treated cats and frogs.

*Leiurus quinquestriatus, Buthotus minax and Parabuthus hunteri* produced rapid, temporary and tachyphylactic contractures in isolated toad sartorius muscle. Tubocurarine has no effect on venom response.

These workers suggested that a protein-like constituent of scorpion venom produced effects on the skeletal muscle fibre resembling those of citrate, lack of calcium or veratrine and these effects could be diminished by the addition of calcium. They postulated that venom administration resulted in the lack of formation of a non-ionized complex with calcium.

Ultrastructural studies of frog sartorius muscle with *Leiurus quinquestriatus* venom suggested its direct action on the frog muscle membrane altering the calcium influx (Karaas and Heirtzler, 1972).

Roasi, Ferreira, Faiva and Santos (1974) found degeneration of muscle fibres in cockroach injected with *Tityus serrulatus* venom.

Ismail, Osman and El-Asmar (1973) suggested a gradual increase in the
amplitude of rat isolated phrenic-nerve hemidiaphragm with Buthus milax venom. The venom produced a contracture in the isolated frog rectus abdominis muscle; this was blocked by d-tubocurarine. They postulated that this might be due to a slow depolarization of excitable membrane of nerve and muscle possibly due to an increase in the sodium permeability or a delay in the inactivation of sodium.

Pandinus exitalis venom reduced the twitch height in cat tibialis anterior muscle preparations following a preliminary increase (Ismail, Osman, Gumaa and Karrar, 1974).
SMOOTH MUSCLE

A number of workers have reported on the action of scorpion venom on several smooth muscle preparations (del Pozo, 1956; Diniz and Goncalves, 1956; Diniz and Valori, 1956; Touitou-Depritre, 1968; Diniz, 1971; Ismail, El-Azzam, Osman, and Ibrahim, 1972; Ismail, Osman, and El-Azzam, 1973; Cunha, Freire-Maia, Tafuri and Maria, 1973 and Ismail, Osman, Gumaa and Karrar, 1974). Their findings in respect of the different venoms have been summarised below.

In vitro effects on intestinal smooth musculature:

Ismail, Osman and El-Azzam (1973) found that *Buthus aonyx* venom contracted isolated rabbit intestine more than guinea-pig ileum. Contractions were decreased markedly by atropine but not by ganglion-blocking agents. They suggested that venom acted by stimulating both branches of autonomic nervous system, and release of acetylcholine.

Ismail, Osman, Gumaa and Karrar (1974) showed that *Pandinus exitialis* venom first relaxed and then contracted rabbit duodenum. Tolazoline and propranolol had no effect on relaxation but the subsequent contraction was blocked by nicotine. The venom produced a contraction in guinea-pig ileum which was blocked by nicotine and atropine. The inhibiting pattern of the venom suggested its action was mediated through the stimulation of adrenergic receptors and also through the stimulation of ganglions.
Atropine raised the threshold for contractile response of isolated guinea-pig ileum and rabbit intestine by Centruroides sp., Scorpio maurus and Tityus serrulatus venom.

Diniz and Gonsalves (1956) reported that venom of Tityus serrulatus and Tityus bahiensis venom produced a sustained and strong contraction of guinea-pig ileum after a latent period. Normal tonus returned after 2-3 minutes and several washings were necessary. Higher concentrations, when repeated, produced tachyphylaxis.

Chymotrypsin incubation abolished the contractile activity of the venom. The activity of the alcoholic extract (but not that of whole venom) was readily dialysable, Atropine reduced the contractile response of gut, but antihistaminics did not. Cocaine blocked the action of the scorpion venom, but was without effect on the action of the acetylcholine. These workers reported that high doses of tubocurarine (5 x 10^{-6} and 6 x 10^{-5}) depressed the action of 5-HT or histamine but had no effect on venom except for a slight delay in time response. They considered that the action of the venom on smooth muscle was similar in many ways to the action of serotonin, both of which were inhibited by atropine and cocaine but differed in their concentration and they could be separated by paper electrophoresis. They postulated that the venom acted on the same structures that were affected by serotonin.

Diniz and Valeri (1959) found that the purified extract of Tityus
serrulatus toxin contracted isolated guinea-pig ileum, rabbit duodenum, rat jejunum and colon. They found that the time for recovery of atropinised preparations were approximately the same for acetylcholine and venom. Hexamethonium iodide had no effect on the contractile activity. In rabbit duodenum bromlysergideethylamide (BOL) had no effect on the toxin activity except an increase of the latent period before the contractile response. This contractile effect lasted long, was apparently venom dose-dependent. Morphine reduced the response to venom, but not to acetylcholine. This reduction in response gradually disappeared with repeated washing. Prior ecarine treatment enhanced the contraction induced by venom on guinea-pig ileum. They suggested that venom acted on cholinergic nervous structures of the entericplexuses.

Tityus serrulatus venom produced a slow contraction of isolated rat ileum preparation after a latent period of 10-60 seconds. Following the slow response the muscle showed spasmodic contractions which lasted for 2-4 hrs, even after repeated washes with bathing fluid. As this effect could be abolished with atropine and potentiated by ecarine, they thought the effect might be due to continuous release of mediator (s) possibly acetylcholine. In half of the experiments, reinforcement of pendular movements and appearance of rhythmic contractions were observed, which according to them, could be due to release of substance P. Subsequent exposure to Tityus toxin produced tachyphylaxis which could be due to inability of the ileum to release active substance (s) for the action of acetylcholine and substance P were not abolished. In venom-treated ileum, 5-HT
response was either blocked or smaller. Methysergide, with or without atropine could not block the effect of the venom, but cocaine did. Nicotine relaxed toxin-treated ileum, an action blocked by alpha and beta adrenergic blockage agents and pentolinium.

Furthermore they found that *Tityus* toxin contracted non-atropinised rat duodenum but relaxed atropinised preparation; this latter response was abolished by pretreatment with phentolamine and propranolol.

Rat uterus was reported to be practically insensitive to *Tityus serrulatus* venom, but this venom increased the sensitivity of the uterus to acetylcholine, serotonin and bradykinin (Diniz and Valeri, 1959). *Leiurus quinquestriatus* venom produced a marked increase in the frequency and amplitude of contraction of the spontaneously motile uterus (Husam, Ismail, El-Asmar and Ibrahim, 1972). This stimulant action was not blocked by atropine, hexamethonium, cocaine, methysergide or bromlysergic diethylamide, while papaverine partially reduced it. However, meclofenamic acid completely blocked the action of the venom on the uterus indicating that the venom might produce its action through the release of kinins.
ENDOGENOUS SUBSTANCES:

The release of endogenous substances by scorpion venom had been suggested by different workers. 

Acetylcholine (Ach):

Diniz and Torres (1968) showed the release of acetylcholine-like substances from guinea-pig ileum by scorpion venom.

Similarly, Tazieff-Depierre (1972) found the spasmogenic action of Androctonus australis toxin to be due to release of acetylcholine which they thought was a calcium-dependent process.

Gomez, Dai and Diniz (1973) reported increased concentration of free acetylcholine and reduced amount of bound acetylcholine after in vitro incubation of rat cerebral cortex with purified toxin of Tityus serrulatus. The release of Ach was dependent on pH, incubation time, an energy source and the concentration of toxin. They found that the effect of Tityus toxin was dependent on the presence of sodium and calcium ions in the incubation medium. Hexamethonium and hemicholium reduced the effect of toxin. Tetrodotoxin blocked the stimulation caused by Tityus toxin.

Catecholamines:

Several workers studied the effects of scorpion venom on adrenal catecholamines (Henriques, Gazzineili and Diniz, 1968; Ganor and Weismann, 1969; Moss,

Gueron and Weizmann (1969) reported an increase in urinary vanillyl mandelic acid, and total free epinephrine and nor-epinephrine following envenoming with Buthus quinquestriatus sting which they contributed to the clinical and cardiovascular manifestations of scorpion sting.

Moss, Colburn and Kopin (1974) studied the release of calcicholamines by Leiurus quinquestriatus venom from rat brain synaptosomes; such release was affected by calcium, duration of exposure and toxin concentration. The authors suggested that the toxin-induced release was similar to that produced by veratrine or tyramine and was distinct from the effects of reserpine.

Kinins:

Scorpion venom stimulated smooth muscle had been suggested to be due to the release of Kinin (Osman, Ismail, and El-Asmar, 1972). The stimulant effect of Leiurus quinquestriatus venom was inhibited by meclofenamic acid on isolated rat uterus. This suggested that the venom might exert its action through the release of kinins. These data seemed to be inadequate for the support of a definite conclusion in this regard.

Pandinus exitialis venom stimulated rat uterus. This action was greatly attenuated by methysergide and completely blocked by meclofenamic acid. These findings led them to suggest that contraction was due partly to venom serotonin contents and partly to release of kinins, prostaglandins and/or slow reacting substances.
Several workers examined the effects of scorpion venom on histamine release and inactivation. Ismail and Osman (1973) found that venom increased significantly the histamine forming capacity of rat stomach \textit{in vivo} but not \textit{in vitro}. They observed no changes in endogenous histamine nor was any alteration of histaminase (diamine-oxidase) activity of the rat intestine.
Hematological Alterations:

Blood Electrolytes:

Mohamed, Robaya and Zaky (1954) found increased and decreased levels of potassium and sodium respectively. They suggested that adrenal cortex was inhibited and that led to alteration in cellular permeability and permit escape of potassium.

Coagulation:

The action of different scorpion venom had been studied on coagulation of blood. Coagulant activity had been found in the venom of Buthus arenicola and Scorpio maurus (Weissman and Shulov, 1959). On the other hand, anticoagulant activity had been reported in Leiurus quinquestratus venom by Todd (1909) and Houssay (1928), and anticoagulant properties partially separated by DEAE Sephadex column chromatography. They showed promotion of factor activation and interference with the action of thrombin upon fibrinogen by different factors of P. gravisiceps venom. Subcutaneous injection of Buthus temnus venom disseminated intravascular coagulation in dogs; this was prevented by heparin.

Hemolysis:

Hemolytic activity of different species of scorpion venom had been reported by a number of workers.
Iltyus bahiensis and Serrulatus venom contained haemolysins, haemorrhagins and leucoctoylcsins and agglutinins (de-Magalhaes, 1925).

The venoms of Buthus pachyrus and Palamneus longimanus had a powerful haemolytic action, those of B. tamulus, F. leucomelas, F. swammerdami and P. xanthopus, a fairly potent one and those of B. alticola, Isctctrus europaeus, F. calupas, F. grevimans, and F. phipsoni had weak hemolysis action.

Balozet (1951) reported that the hemolytic activity of the venoms of Scorpio maurus and snake venom was similar while A. australis venom lacked haemolytic activity. Balozet suggested that the venom contained a phosphatidase which attacked lecithins producing a haemolytic agent.

Buthus quinquemariatus venom was found to have haemolytic activity on human erythrocytes (Mohamed, Rohayem and Zaky, 1953). Human serum inhibited haemolysis of human erythrocytes.

Blood sugar levels:

In experimental animals, the hyperglycemic effect of different species of scorpions had been reported by several workers.

Mohamed (1942, 1950) reported sustained, dose-dependant hyperglycemia with Buthus quinquemariatus venom in experimental animals. In immunized animals where the toxin had been previously incubated with antitoxin the hyperglycemic response was absent.
Liver and muscle glycogenolysis and increased glycogen deposition in rats (Mohamed, Ayobe, Beahkha-resen and El-Damary, 1972); increased release of free fatty acid and lipolytic activity was also demonstrated with venom in _in vitro_ with isolated liver slices but not in epididymal fat pads.

Nair and Kurup (1973) had reported hyperglycemia in experimental rabbits with _Heterometrus scamber_ venom. By estimating vanillyl mandelic acid in paper chromatography they suggested that hyperglycemia in experimental animals was due to the increased production of adrenaline.

El-Asmar, Soliman, Ismail and Osman (1974) examined the effects of adrenergic blockers on the hyperglycemic effect of _Buthus minax_ venom. They showed that pre-treatment with Alpha-blocker (tolazoline) conferred protection against the hyperglycemic effect of this venom but propranolol (Beta-blocker) did not. Since they maintained that _Buthus minax_ released catecholamines (Ismail, Osman and El-Asmar, 1973), they attributed the effectiveness of tolazoline in blocking of the alpha-adrenergic receptor in the liver.
HISTOPATHOLOGICAL CHANGES

Bertke and Atkins (1964) showed that after intramuscular injection of Centruroides sculpturatus venom to rats, there was varying degrees of degeneration, necrosis, edema, and petechial hemorrhages in parenchymatous organs; spleen and lungs and adrenal glands were markedly hypertrophied. Hemorrhage was found in adrenal medulla which extended to zona reticularis and zona fasciculate. In venom-treated animals adrenal ascorbic acid content was decreased, by 4-24 hr, followed by return to normal. They ascribed mortality to adrenal insufficiency.

Mohamed, Saleh, Ahmed and Bashir (1978) studied histopathological effects of Buthus ophisaurus venom on the liver, suprarenal gland and pancreas of mice. They found degenerative changes in the sinusoidal capillaries and extravasation of blood in the zona reticularis and adjacent medulla. Cells of the zona glomerulosa and fascicula had become paler and more vacuolated than those of the control. They showed inhibition of succinic dehydrogenase and increased alkaline phosphatase activity in degenerated hepatic cells. In pancreas, congestion and hemorrhage due to rupture of some large blood vessels were observed in the central part of the pancreas. They showed decreased alkaline phosphatase activity in alpha cells compared to control.
SECTION - 5

CLINICAL FEATURES IN MAN
The clinical features of scorpion sting may vary according to species, size and susceptibility, quantity of venom injected (which varies according to the number of stings), season, age of scorpion, virulence of the venom which depends on the species of scorpion (Sargent, 1947).

The common symptoms are: local swelling, local burning pain. Most generalized symptoms are: hypertension, profuse perspiration, pulmonary oedema, sweating, pallor, lacrimation or restlessness, anxiety, confusion and clouding of consciousness, respiratory depression — dyspnoea, cyanosis, hiccup, hiccoughs, hiperexitation, nausea, vomiting, abdominal pain and cramps, thirst, chest pain, headache, a choking sensation, muscular twitching, hypothermia or pyrexia with or without delirium, hemoptysis, exaggerated reflexes or even arreflexia, excitement, hyperirritability, dizziness, hyperglycemia, glycosuria, shivering, tremor, convulsion and development of a shock-like state of collapse (Gaius and Nheaker, 1932; Sundram, 1932; de negalmae, 1933; Waterman, 1933; Basu 1939; Kent and Stanlake, 1939; Ernst, 1949; Goelke, 1949; Mandel, 1963; Poon-King, 1963; Yaron, 1970; Yaron and Braun, 1970; Yaron, Geron and Braun, 1970).

These clinical features have been described together, but they embrace venoms from all species, so that only some of them occur in envenomation by a given species. Among all others the most important symptoms are related to cardiovascular, respiratory and nervous systems.
CARDIOVASCULAR SYSTEM:

The common features are hypertension, bradycardia sometimes followed by tachycardia, altered heart rate and rhythm, muffled sound, and electrocardiographic changes, suggesting myocarditis and myocardial injury, cyanosis and pulmonary oedema (Sundram, 193; Basu, 1931; Frati, 1949; Maedle, 1961; Poon-King, 1963; Bose, Sarkar, Samorjee and Das, 1966; Aursman and Reeler, 1967; Gueron, 1967; Jain, Chhabra, Shah and Sehah, 1970 and Zarom, 1970).

In scorpion poisoning in Israel, hypertension, tachycardia/bradycardia, both vasoconstriction/vasodilatation and cyanosis are common (Frati, 1949). Frati has reported only the hypertension whereas Zarom (1970), also from Israel, reported the early rise of blood pressure which returned to near normal level after few hours.

In West Bengal, however, scorpion bite generally followed a fall in blood pressure, a situation in contrast with the hypertension found in many parts of the world (Bagchi, personal communication, 1979).

Myocardial damage has also been reported by several workers. Gueron (1967) reported severe myocardial damage in five cases. Poon-King (1963) reported that three-fourths of his patients after a sting of Trinidad scorpion *Tityus trimonistus* evinced electrocardiographic evidence of myocarditis. After a sting of this "black scorpion" in Central India (species not mentioned), E.C.G. changes...
were suggestive of a myocardial injury (Jain, Chhabra, Shah and Sepaha, 1970). Pulmonary oedema after scorpion sting has also been reported by others (Sundararaj, 1931; Menéde, 1964; and Jain, Chhabra, Shah and Sepaha, 1970) and stated to the cause of the death by Sundararaj (1931) though irreversible peripheral circulatory failure has been invoked by Bose, Sarkar, Banerjee and Das (1966).

RESPIRATORY SYSTEM:

As early as 1949 Efriati quoted Sargent (1939) saying that polypnoea is a common feature in twenty two cases of scorpion poisoning.

Patterson (1964) and Yaron (1970) reported respiratory arrest, dyspnoea and cyanosis with a species of Cheliferian scorpions. Caius and Mhasker (1932), while reporting the cases of scorpion sting of Buthidae sp. have mentioned the disturbances in breathing, terminating in respiratory paralysis. Others also mention dyspnoea and hurried shallow respiration (Basu, 1939; Menéde, 1964) and respiratory failure (Das, 1967).

NERVOUS SYSTEM:

Scorpion sting causes paralysis in skeletal muscle. A transient paralysis and paresis of respiratory muscles (Caius and Mhasker, 1932). Several workers (Waterman, 1938; Parsee and Mhasker, 1966; Lath and Bhattacharyya, 1949) have reported hemiplegia.

Efriati (1949) have reported myoclonic twitches and spastic speech.
Defecation and involuntary urination has also been mentioned (de Magalhaes, 1938; Kent and Stahnke, 1939). Speech disturbances mimicking papillary disturbance, ocular myosis, mydriasis, nystagmus, ptosis, exophthalmus, and pupillary areflexia have been reported after scorpion sting (de Magalhaes, 1938; Efrati, 1949).

Waterman (1938) has been reported excretion of scorpion venom in the milk of nursing mother with consequent danger to the infant.
SECTION - 6

INFORMATION AVAILABLE ABOUT THE VENOM
OF THE SPECIES _H. bengalensis_
Hag Chowdhuri (1976), working in this laboratory, reported (1976) about the pharmacodynamic activity of the crude scorpion venom (*Heterometrus bengalensis*) on different physiological systems. A brief account of his findings is given below:

Intravenous *Heterometrus bengalensis* venom (0.1 mg/kg) produced a temporary fall of cat blood pressure, irrespective of the speed of infusion. There was no tachyphylaxis. Venom (0.1 mg/kg) did not alter the hypotensive response of acetylcholine, histamine or 5-hydroxytryptamine, the hypotensive response was not blocked by atropine, mepyramine, or cyproheptadine in doses adequate to block the respective agonists (ACh, His. and 5-HT). Spinal transection did not block the hypotensive response, nor did venom affect significantly carotid occlusion pressor response or preganglionically-stimulated cat nictitating membrane contraction. On cat heart *in situ*, venom (0.4 mg/kg) increased the auricular contraction. On isolated guinea-pig heart amplitude was found to increase while rate remained unchanged.

On isolated rabbit heart, while venom (0.2 mg) produced a slight increase in amplitude of contraction the rate decreased slightly. Coronary outflow was reduced. On isolated guinea-pig auricle amplitude was increased while rate remained unaltered with a venom concentration of $8 \times 10^{-5}$. In rat (venom 4 mg/kg), ECG showed marked bradycardia and auriculo-ventricular dissociation. Venom (0.1 mg and 0.2 mg) produced vasoconstriction in rat hind quarter perfusion and a contractile response in isolated rabbit aortic strip ($1.5 \times 10^{-6}$). Intradermal injection (0.1, 0.2, 0.4, 0.6 and 1.0 mg/0.1 ml venom) increased capillary permeability in rabbit.
Nag Choudhuri (1976) suggested that fall of blood pressure was due to peripheral action of the venom rather than central. Venom-induced hypotension was not blocked by the classical blockers of acetylcholine, histamine or 5-hydroxytryptamine. This was taken to indicate that the autacoids per se were not the cause of hypotension.

In cats there was a decrease in the rate of respiration with 0.16 mg/kg venom. In rats (upto 1.28 mg/kg) and guinea-pigs (upto 0.64 mg/kg) respiratory rates were stimulated but the rate became lowered with higher dosage and produced apnoea, these were absent in bilateral vagotomized rats.

The action of crude venom on different smooth muscle was examined. There was no tachyphylaxis. Venom (concentration g/ml given in parenthesis) contracted isolated smooth muscles in presence of atropine ($10^{-7}$) and cyproheptadine ($10^{-8}$) in the following tissues: guinea-pig ileum ($2 \times 10^{-8}$), guinea-pig tracheal chain ($3 \times 10^{-6}$), rat ileum ($2.5 \times 10^{-6}$), rat fundus strip ($2.8 \times 10^{-6}$), rat ascending colon ($1.7 \times 10^{-6}$), rat duodenum ($6 \times 10^{-6}$), rat uterus ($6 \times 10^{-6}$), hen rectal caecum ($6 \times 10^{-6}$), human myometrium ($10^{-7}$), goat tracheal chain ($10^{-7}$), and isolated rabbit duodenum ($1.2 \times 10^{-6}$). Isolated guinea-pig was different was not contracted up to concentrations of $10^{-4}$.

Simple physiochemical parameters were examined on the contractile effect of the venom, as tested on guinea-pig ileum. Contractile activity was found to be heat-stable; however, alkali-boiling of the venom produced a significant loss (42.9%) of contractile activity. Contractile activity
remained unaltered on incubation with trypsin (1 mg per mg venom) and chymotrypsin (2 mg per mg venom).

Gocalne ($10^{-4}$), morphine ($10^{-4}$), pentolinium ($10^{-6}$), ibuprofen($10^{-4}$), ketoprofen ($10^{-4}$), and dehydroemetine ($10^{-6}$) did not show any antagonism to the venom induced contractile activity in guinea-pig ileum.

Parallel bioassay of venom carried out against acetylcholine, histamine, 5-hydroxytryptamine and bradykinin on different smooth muscle preparations showed high indices of discrimination which distinguished venom contractile activity from the aforementioned agonists.

In rat phrenic nerve-diaphragm preparation venom ($4 \times 10^{-5}$) did not alter the amplitude of contraction induced by nerve stimulation or d-tubocurarine ($5 \times 10^{-5}$) induced relaxation. With toad rectus abdominis preparation, venom ($4 \times 10^{-5}$, $4 \times 10^{-6}$, $2 \times 10^{-6}$) did not produce any contraction, but in lower doses ($4 \times 10^{-7}$), it increased the acetylcholine-induced contraction and in higher doses ($2 \times 10^{-6}$) inhibited acetylcholine-induced contractions.

Venom could not be shown to release histamine and 5-HT, at respective concentrations (of venom) of $5 \times 10^{-7}$ and $3 \times 10^{-6}$.

LD$_{50}$ in male mice was 11.52 mg/Kg. Crude scorpion venom ($5 \times 10^{-6}$) did not show any haemolytic activity. Venom ($1.1 \times 10^{-3}$) inhibited human plasma cholinesterase (GNE) activity, but not rat brain GNE activity.
Venom dialysate \(10^{-7}\) produced a contractile response on guinea-pig ileum which was resistant to atropine \(10^{-7}\) and cyproheptadine \(10^{-8}\). Incubation with trypsin (1 mg per mg venom) produced some reduction in contractile activity of dialysate. Incubation with chymotrypsin (4 mg per mg) and papain (1 mg per mg venom equivalent) did not have any significant action. Heat treatment did not lead to any significant loss of dialysate contractile activity. Acidification and acid heating did not produce any loss of dialysate contractile activity while alkalinisation and alkali heating led to significant loss of dialysate contractile activity.

On intravenous injection, dialysate produced a hypotensive effect in cat; the dialysate did not alter blood pressure. This produced pain in mice but the dialysate was devoid of such activity.