SOME PROPERTIES OF THE CRUDE EXTRACT OF SCORPION TELSONS' FROM *HETEROMETRUS GRAVIMANUS* AND PRESENCE OF ACETYLCHOLINE- AND HISTAMINE-LIKE SUBSTANCES

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The lyophilized crude scorpion venom from *Heterometrus gravimanus* was administered to anesthetized cats, and the changes in electrocardiogram, hypotensive effect, increase in the depth of respiration and blood flow were observed. From the pharmacological experiment on isolated guinea pig ileums, the presence of both acetylcholine- and histamine-like substances was suggested in the crude venom. Acetylcholine-like substance was compared with an authentic acetylcholine on the silicagel thin-layer chromatography, and the amount was estimated to be 8—15 μ g equivalent to acetylcholine per mg venom by the Magnus method. Histamine-like substance was extracted and purified from the crude venom, and was run on both silicagel and polyamide thin-layer chromatographies concurrently with an authentic histamine and 5-hydroxytryptamine. By both bioassay with isolated guinea pig ileum and chemical determination with the fluorometric methods, the content of histamine-like substance was calculated to be about 30 μ g equivalent to histamine per mg venom. 5-Hydroxytryptamine was not found. Hemolytic action was observed in the human erythrocytes with the venom and the maximal action was found at pH 5.5.

Keywords—*Heterometrus gravimanus* scorpion; lyophilized crude scorpion venom; crude extract of scorpion telsons' from *Heterometrus gravimanus*; acetylcholine-like substance; histamine-like substance; hypotensive action; contraction of guinea pig ileum

INTRODUCTION

Some species of scorpion (*Lincholes australiasiae* FABRICAS, *Isometrus europeus* LINNE and *Buthus martensii* KARSCH) are found in some parts of Far East Asia, that is, Taiwan, Korea and China. *Heterometrus gravimanus* is a species found in India.

It is said that the venom of oriental scorpion is remarkably less toxic than that of the scorpions of occidental species, especially tropical ones, because

only a few people die of the sting by oriental scorpions. However, the research concerning the venom of oriental scorpions have not hitherto been made, except a few investigations.¹⁻³⁾

The pharmacological and biochemical investigations of the crude venom from *Heterometrus gravimanus* have revealed the presence of high content of substances such as acetylcholine- and histamine-like substances in the venom. In this report, some biochemical observations are given

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followed by the description of acetylcholine- and histamine-like substances from the crude venom of *Heterometrus gravimanus* species.

MATERIALS AND METHODS

Lyophilized Crude Venom—*Heterometrus gravimanus* specimens were collected in the vicinity of Agra, India, and telsons part which contain venom gland and venom were cut off. The crude extract was obtained by maceration of crushed and ground telsons in distilled water (10 ml for 1 g of ground powder). After centrifugation at $10000\times g$, at 0° for 10 min, the supernatant was separated and lyophilized (0.34 g of lyophilized extract from 1 g of a raw material). In this report, the lyophilized extract is referred as the crude venom.

Pharmacological Methods

Estimation of Toxicity—The toxicity (LD_{50} at 48 hr) of the lyophilized crude venom was estimated in albino mice (ddY) weighing 15 to 20 g by *i.v.* injection (tail vein) of an aliquot of the venom dissolved in saline containing bovine serum albumin (final concentration: 2 mg per ml).

Methods in Anesthetized Cats—A total of 3–4 healthy cats of either sex weighing 2 to 4 kg, were employed in these experiments. All cats were anesthetized with 30 mg per kg of sodium pentobarbital administered *i.p.* The degree of anesthesia was maintained constant throughout the experiment by giving small maintenance doses of the anesthetic when necessary.

The systemic blood pressure was recorded from the right common carotid artery *via* a pressure transducer (Nihon Kohden, MP-5S, Japan) and the blood flow was recorded from the left common carotid artery *via* a transducer (Cuff type, lumen size 1 mm, Nihon Kohden, FA 010T, Japan) on a multiple purpose polygraph (Nihon Kohden, Model RM-45, Japan).

Electrocardiogram (Lead II) and respiration (with elastic rubber tubing) were recorded simultaneously on the same polygraph.

Methods in Isolated Guinea Pig Ileum—The ileum from guinea pig (about 350 g) was cut into strips of approximately 3.0 cm and suspended

(vertically) in a 30 ml bath containing (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.4, $MgCl_2$ 0.5, NaH_2PO_4 0.3, $NaHCO_3$ 11.9 and glucose 5.6 (solution pH 7.8). The medium was maintained at $27^\circ \pm 1^\circ$ and continuously oxygenated with a mixture of 95% O_2 –5% CO_2 (v/v). The strips were fixed to the recorder to yield approximately eight-fold amplification.

Biological Estimation of Acetylcholine- and Histamine-like Substances—The isolated guinea pig ileum strips were allowed to equilibrate for 1 hr before responses were tested. The antagonists, atropine (10^{-5} M) or diphenhydramine (10^{-4} M) were present in the Tyrode solution throughout the experiments.

Cumulative technique was employed and response curves were determined by adding 0.1 ml of solution of the lyophilized crude venom or extracted histamine-like substance from the crude venom in the absence and presence of atropine (10^{-7} M) or diphenhydramine (10^{-8} M), respectively. After the maximal response was obtained, the strips were washed until the developed tension return to the control value.

Acetylcholine chloride and histamine dihydrochloride were used as the authentic agonists, and all drug concentrations were expressed in terms of the weight of the base. Control contractions to each agonist were obtained at 1 hr intervals.

The pD_2 - and pA_2 -values were calculated from the cumulative dose response curves as described by Van Rossum.⁴⁾ The amount of acetylcholine- and histamine-like substances in the crude venom was expressed in weight equivalent to authentic acetylcholine and histamine, respectively.

Chemical and Biological Methods

Thin-Layer Chromatography—Chromatography of the lyophilized crude venom was performed on both silicagel (Kiesel gel 60, F₂₅₄, E. Merck, West Germany) and polyamide layer (Cheng Ching Trading Co., Taiwan) at room temperature.

The solvent system used for developing chromatogram of histamine and 5-hydroxytryptamine was 14% (w/v) sodium chloride in 25% (v/v) acetic acid solution, and for the chro-

matogram of acetylcholine either the same solvent above or *n*-butanol–ethanol–acetic acid–water (8:2:1:3, v/v) was used. Ascending chromatograms were developed, and the layers were dried and sprayed with the color reagent. Pauly's reagent (sulfanilic acid 1% (w/v) in 10% (v/v) HCl followed by 5% (w/v) sodium carbonate and 20% (w/v) NaCl) was adopted for the detection of histamine and 5-hydroxytryptamine, and an iodide-platinum reagent (1 g of platinum chloride in 100 ml of 2% (w/v) potassium iodide solution) for acetylcholine.

Extraction of Histamine-like Substance—The lyophilized crude venom was homogenized with 0.4 N perchloric acid with Teflon homogenizer under cooling, and then the homogenate was centrifuged at $10000\times g$ for 30 min at 0° (Automatic high speed refrigerated centrifuge, Hitachi, Model 18 PR-3, Japan). To the supernatant, 0.5 M phosphate buffer (pH 7.5) was added, adjusted the pH to 7.5 with 5 N KOH, and centrifuged at $3000\times g$ for 15 min at 0° .

The supernatant was applied to a Bio Rex 70 column (7×85 mm) which had been equilibrated with 0.5 M phosphate buffer (pH 7.5). The column was eluted stepwise with 0.5 M sodium acetate buffer (pH 6.0) followed by 0.5 N sodium hydroxide solution and finally with 1.0 N aqueous sodium hydroxide. Histamine-like substance was eluted with a 0.5 N sodium hydroxide solution and was transferred successively to *n*-butanol, *n*-heptane and water as described by Shore,⁵⁾ and then the water layer was lyophilized.

Chemical Determination of Histamine-like Substance—The extracted histamine-like substance was estimated by the fluorometric method. Both *o*-phthalaldehyde^{5,6)} and fluorescamine⁷⁾ were used as the fluorogenic reagents. The intensity of fluorescence was measured with a fluorescence spectrophotometer (Hitachi, Model 204, Japan) at the following wavelengths: *o*-phthalaldehyde (activation; 360 nm and emission; 450 nm) and fluorescamine (activation; 400 nm and emission; 475 nm). Reagents blanks were run through the reaction and all fluorescence values have been corrected for blanks.

Esterolytic Activity—This activity was estimated by the spectrophotometric method as described before⁸⁾ using both *N*- α -tosyl-L-arginine methyl ester and *N*- α -benzoyl-L-arginine ethyl ester as the substrate at 25° , pH 8.0.

Hemolysis by the Lyophilized Crude Venom—Citratd human blood was kindly supplied by Hokkaido Blood center of Japanese Red Cross Society. The blood was centrifuged for 15 min at $1500\times g$ and the cells were washed three times with isotonic saline (pH 7.4). The washed, packed cells were suspended in isotonic saline or buffered isotonic saline (pH range 5–8) to yield suspension of 10% (v/v) cell concentration. An equal volume of the test solution (20 mg of the lyophilized crude venom in isotonic saline) was added quickly to 2 ml of the cell suspension in test tubes.

The mixtures were then incubated for 1 hr at 37° with shaking and then centrifuged at $1500\times g$ for 15 min to remove the unhemolyzed cells. The supernatant was collected and the degree of hemolysis was determined by estimating the absorbancy of hemoglobin (at 543 nm) released in the supernatant liquid with a spectrophotometer (Model 200-20, Hitachi, Japan).

Chemicals—Acetylcholine chloride (Sigma, U.S.A., Daiichi Pharmaceutical Co., Japan), atropine sulfate (Sanko Pharmaceutical Industries, Japan), *N*- α -benzoyl-L-arginine ethyl ester (Protein Research Foundation, Japan), Bio Rex 70 (Bio Rad Laboratories, U.S.A.), bovine serum albumin (Miles Laboratories, U.S.A.), diphenhydramine (Kowa Pharmaceutical Co., Japan), fluorescamine (Japan Roche), Heparin (Wako), histamine (free base, Wako), histamine dihydrochloride (Nakarai Chemicals, Japan), 5-hydroxytryptamine creatine sulfate (Sigma), *o*-phthalaldehyde (Sigma), sodium pentobarbital (Tanabe Pharmaceutical Co., Japan), and *N*- α -tosyl-L-arginine methyl ester (Protein Research Foundation).

RESULTS

Anesthetized Cats

The lyophilized crude venom was administered by injecting *i.v.* to three cats. A dose of 0.05 mg per kg had no observable effects on the elec-

FIG. 1. *Electrocardiogram, Blood Pressure and Flow, and Respiration following Administration of the Lyophilized Crude Venom from Heterometrus gravimanus* Male cat (3.6 kg) under pentobarbital anesthesia. Lyophilized crude venom, 0.5 mg per kg, administered intravenously. Records taken over a period of 30 min.

trocardiograms, blood pressure, respiration and blood flow. Doses of 0.2 and 0.5 mg per kg produced hypotension that started 0.4–0.7 min after the injection, reaching the maximum 0.7–1.25 min later and the effect disappeared within 30 min (Table I). There was a decrease in the diastolic pressure with significant changes in the pulse pressure (Fig. 1).

Changes in the electrocardiogram after the administration of doses of 0.2 and 0.5 mg per kg showed an apparent decrease in the amplitude of QRS complex and depressed S-T segment. An increase in the blood flow was also observed. The respiratory effect of the venom consisted of an increase in the depth of respiration with a simultaneous hypotensive effect. The hypotension was partially blocked by the treatment with diphenhydramine (0.1 and 0.5 mg per kg) but not with atropine (0.1 and 0.5 mg per kg).

Isolated Guinea Pig Ileum

The lyophilized crude venom (10^{-7} g per ml)

TABLE I. *Vasodilator Activity after Administration of the Lyophilized Crude Venom and Venom Plus Antagonists*

Cats	Dose (mg/kg)	Arterial pressure ^{a)} (mm Hg)		Onset of fall (min)	Time of maximal fall (min)	Return to initial pressure (min)	Extent of diastolic fall (mm Hg)	% of maximal fall
		Control	Administ.					
21106K	Venom (0.2)	150/129	150/62	0.42	0.67	28.1	56	37
	+ DPH ^{b)} (0.1)	150/90	147/60	0.43	1.25	28.0	48	34
21108K	Venom (0.2)	148/116	136/72	0.73	1.00	10.2	44	34
	+ Atr. ^{c)} (0.01)	168/124	124/74	0.50	1.00	10.2	50	40
	+ DPH ^{b)} (0.1)	160/116	152/104	0.50	1.30	38.0	12	10
	Venom ^{d)} (0.5)	150±3.7/ 120±3.7	136±9.9/ 48±2.8	0.50±0.03	0.78±0.07	12.9±2.1	67.0±1.9	58.3±1.8
21227K	+ DPH ^{b)} (0.5)	168/128	144/96	0.50	0.90	4.3	32	25
	Venom (0.5)	160/110	120/44	0.50	1.00	15.0	40	25
	+ Atr. ^{c)} (0.1)	128/88	92/44	0.50	1.00	14.5	36	28
	+ Atr. ^{c)} (0.5)	152/116	128/84	0.80	1.40	15.0	24	16
	+ Atr. ^{c)} (0.5)	152/112	140/96	0.58	0.88	13.0	12	8
	+ DPH ^{b)} (1.0)							
	+ Atr. ^{c)} (0.5)	144/108	132/100	0.75	1.40	4.0	12	8
	+ DPH ^{b)} (1.0)							
	Venom (0.5)	136/96	116/76	0.50	1.30	15.0	20	15
	+ Atr. ^{c)} (0.5)	132/100	124/64	0.75	1.40	15.0	20	15
	+ Atr. ^{c)} (0.5)	128/96	116/80	0.75	1.00	6.0	12	9
	+ DPH ^{b)} (1.0)							

a) At maximal fall.

b) DPH: diphenhydramine.

c) Atr.: atropine.

d) Four experiments; mean±S.E.

FIG. 2. *Contraction of Guinea Pig Ileum with the Lyophilized Crude Venom*
Cumulative log dose-response curves showing the effect of the lyophilized crude venom on degree of the contraction of isolated guinea pig ileum without atropine ($\circ$ —, means and standard errors) and in the presence of atropine ($\bullet$ —, means and standard errors). Each point is the mean of three determinations.

caused an immediate contraction of the smooth muscle (guinea pig ileum). Control experiments were performed with a lyophilized powder of water extract from the skeletal muscle of rats, and no effect was observed on isolated guinea pig ileum. Treatment with both atropine (10^{-7} M) and diphenhydramine (10^{-8} M) reduced contractile effect of the lyophilized crude venom on guinea pig ileum.

Fig. 2 shows a typical cumulative log dose-response curve of the crude venom, with or without atropine as the antagonist. The presence of acetylcholine-like substance was suggested from the inhibition curve. The amount of acetylcholine-like substance in the lyophilized crude venom (per mg) was estimated to be $8\text{--}15\text{ }\mu\text{g}$ (10.80 ± 1.24 ; $M \pm S.E.$, $n=5$) equivalent to acetylcholine in the bioassay.

The cumulative log dose-response curve of the extracted and purified histamine-like substance with or without diphenhydramine is shown in Fig. 3. The amount of this substance was calculated to be $26\text{--}35\text{ }\mu\text{g}$ (31.1 ± 0.73 ; $M \pm S.E.$, $n=9$) equivalent to histamine.

Chemical Determination of Histamine-like Substance

FIG. 3. *Effect of Extracted Histamine-like Substance from Heterometrus gravimanus on Guinea Pig Ileum*
Cumulative log dose-response curves with diphenhydramine as an antagonist ($\bullet$ —, means of three experiments and standard errors) and without antagonist ($\circ$ —, means of three experiments and standard errors) for extracted histamine-like substance from lyophilized venom on the contraction of isolated guinea pig ileum are shown.

Fluorometric methods were carried out using *o*-phthalaldehyde and fluorecamine as the fluorescent agents, and the content of histamine-like substance per mg of the crude venom was calculated to be $30.80\text{--}36.39\text{ }\mu\text{g}$ (33.25 ± 0.73 ; $M \pm S.E.$, $n=9$) and $31.20\text{--}32.87\text{ }\mu\text{g}$ (31.84 ± 0.53 ; $M \pm S.E.$, $n=3$) equivalent to histamine, respectively.

Thin-Layer Chromatography

Thin-layer chromatographies were performed on silicagel plate for the acetylcholine-like substance. The R_f value of authentic acetylcholine was 0.1 when the solvent system of *n*-butanol-ethanol-acetic acid-water was used (8:2:1:3, v/v) (Fig. 4 (a)). The R_f value of acetylcholine has been either zero or very low in all solvents hitherto employed for its chromatography. In the present experiment, however, we have succeeded in obtaining a high R_f value (0.5) in the solvent system of 14% (w/v) sodium chloride in 25% (w/v) acetic acid (Fig. 4 (b)). The color spots were produced as greyish blue in the reddish background. In some cases, the fluorescence was

FIG. 4. Thin-Layer Chromatographies of Acetylcholine and the Lyophilized Crude Venom from *Heterometrus gravimanus*

A 10 μ l and 20 μ l aliquots of acetylcholine solution (2 mg per ml) and the lyophilized crude venom (10 mg per ml), respectively, were spotted on silicagel plates and developed in both solvent systems containing *n*-buthanol-ethanol-acetic acid-water (8:2:1:3, v/v) (a) and 14% (w/v) sodium chloride in 25% (w/v) acetic acid solution (b). Spots circled with the dotted line in (a) showed fluorescent substances under the UV light. A, acetylcholine; V, lyophilized crude venom.

observed under the UV light.

After unsuccessful attempts to separate histamine from 5-hydroxytryptamine by thin-layer chromatography in several solvent systems, a distinct separation of these two substances was achieved by using 14% (w/v) sodium chloride in 25% (w/v) acetic acid. Authentic histamine showed an *Rf* value of 0.9 on polyamide layer and 0.1 on silicagel. On the other hand, the *Rf* value of 5-hydroxytryptamine was 0.35 and 0.75 on polyamide and silicagel, respectively. The lyophilized crude venom and purified histamine-like substance exhibited a good resemblance with the authentic histamine, but not with 5-hydroxytryptamine (Fig. 5 (a) and (b)). Both histamine and 5-hydroxytryptamine showed red color with Pauly's reagent on polyamide layer. However, on

the silicagel, histamine and 5-hydroxytryptamine gave yellowish red and yellowish brown colors, respectively.

Biochemical Properties

The lyophilized crude venom showed poor esterolytic activity, which was estimated to be 0.010 and 0.021 μ moles hydrolyzed per min per mg of the crude venom with *N*- α -benzoyl-L-arginine ethyl ester and *N*- α -tosyl-L-arginine methyl ester as the substrates, respectively.

The crude venom caused hemolysis in human erythrocytes. Fig. 6 showed the pH profile of hemolytic action with the crude venom, and maximum activity was found at pH 5.5.

DISCUSSION

The LD₅₀ of *Heterometrus gravimanus* venom per 20 g albino mice was 1.88 mg, which seemed to be relatively low in comparison with the venoms of other scorpion species. However, the lyophilized crude venom used in this study is the mixture of the scorpion venom plus telson tissue extract, that it is not so obvious that the venom of this scorpion is really low in toxicity. One of the authors¹¹ estimated the LD₅₀ to be 0.0005 mg per g (Albino mice) for the purified toxin (Buthotoxin) from *Buthus martensii* KARSCH. Rochat *et al.*⁹⁾ reported the LD₅₀ of the purified *Androctonus australis* to be 19 μ g per kg for Toxin I and 10 μ g per kg for Toxin II, on 20 g albino mice. However, the comparative potency of various scorpion species per 15 g albino mice (subcutaneous injection) varied from 0.005 mg (*Liorus quinquestriatus*) to 40 mg (*Hadogenes sp.*).¹⁰⁾ The variation in toxicity may be attributable to the species differences and the location where the scorpions were captured.

The clinical effects of scorpion venom from various species are referred to be, especially, hypertension, respiratory failure and stimulation of skeletal muscles.¹¹⁾ Therefore, in our preliminary observations on anesthetized cats, the marked hypotensive effect of *Heterometrus gravimanus* venom was rather unexpected. Furthermore the lyophilized crude venom caused an immediate contraction of smooth muscle on guinea pig ileum. Many substances, *e.g.*, acetylcholine, histamine,

FIG. 5. Thin-Layer Chromatographies on Histamine, Lyophilized Crude Venom, Extracted Histamine-like Substance from *Heterometrus Gravimanus* and 5-Hydroxytryptamine. The solvent system of 14 % (w/v) sodium chloride in 25 % (v/v) acetic acid solution was used for both silicagel (a) and polyamide layer (b).

H, histamine (10 μ l aliquot of 10 mg per ml solution).

P, extracted histamine-like substance from *Heterometrus gravimanus* (10 μ l aliquot of 0.5 mg per ml solution).

M, mixture of authentic histamine (H) and extracted one (P).

S, 5-hydroxytryptamine (serotonin, 10 μ l aliquot of 5 mg per ml solution).

FIG. 6. The pH Profile of Hemolytic Action with the Lyophilized Crude Venom from *Heterometrus gravimanus*.

Blank experiments were run at the each pH point and all hemolyzed values have been corrected for blanks. Each value is the average of duplicate results.

5-hydroxytryptamine and kinins, are known to have the smooth muscle contracting activity. Kinins are illustrated as the slow-acting principles produced by the action of trypsin or snake venom on plasma or a globulin fraction,¹²⁾ having both hypotensive and smooth muscle stimulating action, which are not antagonized by atropine or anti-histamines.¹³⁾ Our experiments showed that the contraction of the isolated guinea pig ileum by *Heterometrus gravimanus* crude venom was antagonized by both atropine and diphenhydramine. So, the existence of acetylcholine- and histamine-like substances was assumed to be in this crude venom. The content of acetylcholine-like substances was assayed by Magnus method with the lyophilized crude venom, and the amount was calculated to be 8–15 μ g equivalent to acetylcholine per mg. The presence of acetylcholine-like substance was also indicated by the test results with or without atropine as an antagonist, and further from the thin-layer

chromatographies. Although, Dinitz and Torres¹⁴⁾ reported the release of acetylcholine-like substance from guinea pig ileum by *Tityus serrulatus* and *T. bahiensis* venom, *Heterometrus gravimanus* is the first crude scorpion venom reported to have acetylcholine-like activity. However, further studies would be required to establish the acetylcholine-like substance to be the true component of a pure venom. It was suggested that in nature, acetylcholine might have some functions other than a neuro-transmitter, and the significance of acetylcholine in the non-innervated tissues has never been explained.¹⁵⁾

Histamine-like substance was extracted from the lyophilized crude venom as described. The result of thin-layer chromatographies showed clearly the presence of histamine-like substance in the crude venom. Fluorometric determinations exhibited that the content of histamine-like substance was about 30 μg equivalent to histamine per mg of the lyophilized crude venom. The fluorescent agents of *o*-phthalaldehyde and fluorecamine were compared with each other in the process of determination, and both reagents were observed to have a similarly good sensitivities to histamine. By the bioassay with guinea pig ileums, the reasonable values were obtained. Ismail *et al.*¹¹⁾ reported the presence of histamine in a South Asian scorpion *Palamneus gravimanus*, which contained only a small amount of venom,¹⁶⁾ and estimated the histamine content to be 5–7.2 μg per mg dry venom by a biological assay. It is interesting to state

that the scorpion venom used in this investigation (*Heterometrus gravimanus*) showed a very low LD₅₀, and contained a histamine-like substance. In this respect, there is a good agreement with the report of Ismail *et al.*¹¹⁾

Table II shows the vascular reactivity after administration of a mixture of acetylcholine and histamine. The response to the mixture of acetylcholine (5 μg) plus histamine (15 μg) is nearly equal to that of the lyophilized crude venom of 0.5 mg per kg. Therefore, administration of antagonists of acetylcholine and histamine were performed simultaneously upon the envenomation of the crude venom in this experiment. Atropine administration (0.01 and 0.05 mg per kg) seemed to have no effect on the arterial blood pressure of cats (Table I). Diphenhydramine had some effects on the envenomation, namely, a delayed onset of maximum fall and a reduction in the extent of diastolic fall were observed. However, the degree of maximal fall varied from animal to animal.

In wasp venom, the presence of histamine, 5-hydroxytryptamine, and a slow contracting substance were reported,¹⁷⁾ and in honet venom, acetylcholine, 5-hydroxytryptamine, histamine and kinin were identified.¹⁵⁾ Adam and Weiss¹⁸⁾ reported the presence of 5-hydroxytryptamine in scorpion *Leiurus quinquestriatus*. Attempts to isolate 5-hydroxytryptamine from *Heterometrus gravimanus* venom by means of an ion exchange resin (Bio Rex 70) were in vain. It was also not detectable on chromatograms developed concur-

TABLE II. Vascular Reactivity after Administration of the Mixture of Acetylcholine and Histamine

Cats	Dose (mg/kg)	Arterial pressure ^{a)} (mm Hg)		Onset of fall (min)	Time of maximal fall (min)	Return to initial pressure (min)	Extent of diastolic fall (mm Hg)	% of maximal fall
		Control	Administ.					
21108K	Venom ^{b)}	150 $\pm$ 3.7/	136 $\pm$ 9.9/	0.50 $\pm$ 0.03	0.28 $\pm$ 0.07	12.9 $\pm$ 2.1	67.0 $\pm$ 1.9	53.8 $\pm$ 1.8
	(0.5)	120 $\pm$ 3.7	48 $\pm$ 2.8					
21227K	Mix. ^{c)}	152/116	136/50	0.46	0.67	17	66	57
	Venom ^{b)} (0.5)	160/110	120/44	0.50	1.0	15	40	25
	Mix. ^{c)}	148/112	112/56	0.88	1.4	8	28	21
	Venom ^{b)} (0.5)	136/96	116/76	0.50	1.3	15	20	15
	Mix. ^{c)}	136/102	108/64	0.88	1.4	8	28	21
	Mix. ^{c)}	120/88	96/56	0.88	1.5	8	24	20

a) At maximal fall.

b) From Table I.

c) Mixture: acetylcholine 5 μg plus histamine 15 μg .

rently with an authentic 5-hydroxytryptamine and the lyophilized crude venom. Further, several experiments on rat uteri showed its absence in the lyophilized crude venom.

Mohamed *et al.*¹⁹⁾ reported the protease activity by using urea denatured hemoglobin in *Buthus quinquestriatus* venom. We estimated the esterolytic activity of the lyophilized crude venom with both N- α -tosyl-L-arginine methyl ester and N- α -benzoyl-L-arginine ethyl ester as the substrates, and a very poor activity was found. This venom seemed to have a small amount of trypsin (EC 3.4.4.1) or trypsin-like substances.

According to the description by Balozet,¹⁶⁾ scorpion venom generally contained a few or no hemolysins except a few species. Indian *Heterometrus* is one of such exceptions. About 90% of the hemolytic action was lost by heat treatment at 80° for 5 min at pH 5.5 (unpublished data). So, the hemolysin(s) in *Heterometrus gravimanus* seemed to be heat-labile and of proteinous in nature. Our investigations on the purification of hemolysin(s) are in progress. Rosin²⁰⁾ reported the hemolytic action of *Nebo hierichonticus* venom, using agar-blood plates of 5 percent washed human erythrocytes.

From these experiments, pharmacological effects of the crude venom on cats were speculated to be mainly due to acetylcholine- and histamine-like substances. However, the possibility that proteins and enzymes are present in the venom other than acetylcholine- and histamine-like substances is not precluded. The polypeptide neuro-toxin(s) which have been found in several scorpion venoms also could be found in the venom of *Heterometrus gravimanus*.

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