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Studies on Cardiotoxin and Vasoactive-Substance-Releasing

Chen-Yuan Lee, M.D.

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Pharmacological Institute. College of Medicine
National Taiwan University
Taipei, Taiwan, China

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ABSTRACT

1. Preparing Institution: The Pharmacological Institute, College of Medicine National Taiwan University, Taipei, Taiwan, Republic of China

2. Title of Report: Studies on Cardiotoxin and other vasoactive-substance releasing component(s) in Cobra venom.
   I. Isolation of cardiotoxin and other vaso-active substance releasing component(s).

3. Principal investigator: Chen-Yuan Lee, M. D.

4. Co-worker: C. C. Chang, T. H. Chiu, P. J. S. Chiu, T. C. Tseng, and S. Y. Lee

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Lyophylized venom of Naja naja atra was fractionated on column of CM-Sephadex (G-50) into 13 fractions by gradient elution with ammonium acetate buffer at pH 5-7. Among them five fractions (V-IX) were found to be neurotoxic and three (X, XII, XIII) were cardiotoxic. Intraperitoneal LD_{50} in mice was 1.074 µg/g for Fr. VIII- the major neurotoxic component (NT) and 1.48 µg/g for Fr. XIII- the major cardiotoxic one (CT). CT caused contracture, as well as reduction of resting membrane potentials, of the frog's sartorius, chick's biventer cervicis, and rat's diaphragm. In the absence of calcium, the contracture was markedly reduced, although the depolarizing effect remained unchanged. Neither contracture nor depolarization was caused by NT. The terminal nerve spikes of the frog

Cont'd

sartorius were abolished by CT but unaffected by NT. CT caused systolic arrest of isolated frog hearts and rat's atria by reducing the membrane potentials, whereas NT was almost without effect up to 10^{-4} g/ml. CT caused a slow contraction of the guinea pig ileum, which was partially antagonized by either atropine or procaine but not by hexamethonium or antihistaminics. In the presense of CT ($10^{-6} - 10^{-5}$ g/ml), the responses to nicotine and 5-hydroxytryptamine were greatly inhibited, usually preceded by an initial and transient facilitation. The responses to histamine and acetylcholine were not, or only slightly, reduced by CT. The vessels of the rabbit ear were constricted by CT. In cats, CT caused a fall in systolic pressure more than diastolic pressure, accompained by various ECG changes, such as P-R interval prolongation, inverted T waves, S-T segment depression, ventricular premature beats, A-V interference, complete A-V block, idioventricular rhythm etc. It is concluded that cardiotoxin isolated from cobra venom acts on various excitable cells, predominantly, if not entirely, by reducing the membrane potentials.

Although the primary cause of death from cobra venom has been shown to be peripheral respiratory paralysis in many species of animals (Kellaway, Cherry & Williams, 1932; Lee & Peng, 1961; Vicks, Ciuchta & Pollay ; 1965), the venom also produces profound cardiovascular changes. When envenomed animals are maintained by artificial respiration they finally die of circulatory collapse. Several active components such as neurotoxin, cardiotoxin, phospholipase A, and some proteins having other enzymatic activities have been separated from cobra venom (for references see Slotta, 1955 and Meldrum, 1965). However, it has not been established as to which component(s) or to what extent these components are responsible for the cardiovascular effects caused by crude cobra venom. While cobra neurotoxin has been isolated in crystalline form (Yang, 1965) and the mode of its neuromuscular blocking action has been studied at length (Su , Chang & Lee, 1966; Chang & Lee, 1966), "cardiotoxin" isolated by Sarker (1947) has been shown not to be a single protein (Raudonat & Holler, 1958) and the mode of its action has not been fully elucidated.

In the investigation to be described below, we have attempted to purify cardiotoxin as pure as possible, and its effects on various kinds of muscles have been studied in detail in order to shed some light on its mode of action.

MATERIALS AND METHODS

Venom The venom of Naja naja atra used in this study was freshly collected and lyophilized in this laboratory and stored in dry state in a vacuum desiccator. Its intraperitoneal LD_{50} in mice (N. L. H. strain) was 0. 44 (0. 40-0. 48) μg/g body weight.

Zone electrophoresis on starch The method of Kunkel and Slater (1952) modified by Fahs-Beck and Li (1954) was followed. The experimental conditions were essentially the same as previously described for Bungarus venom (Chang & Lee, 1963).

Column Chromatography CM-Sephadex* columns were prepared and packed in the manner described by Peterson et al (1962). CM-Sephadex was equilibrated with 0. 005M ammonium acetate buffer, pH 5. 0, and then packed into a column of 1. 6 x 80 cm at 4°C.

The gradient was established by adding 0. 9M ammonium acetate buffer, pH 7. 0, into a flask containing 450 ml of 0. 005 ammonium acetate buffer, pH 5. 0. The flow rate was 7. 5 ml/hr for the first 24 hours and then 5 ml/hr afterwards. The void volume was 3 ml for each tube. The elution pattern was followed by reading the absorption at 280 mμ with Beckman D. U. Spectrophotometer. The eluates corresponding to the same peak were pooled and lyophilized for subsequent study.

Toxicity in Mice** Selected doses of each fraction were injected intraperitoneally into mice weighing 18-20 g. The concentration was so adjusted that

* CM-C-50 of Pharmacia product, medium 4, capacity 7 meq/g

** NIH strain mice were kindly donated by U. S. Naval Medical Research Unit No. 2, Taipei.

the required dose was contained in 0.1-0.2 ml saline per 10 g body weight of mice. LD_{50} was computed according to the method of Litchfield and Wilcoxon (1949).

Diventer cervicis nerve-muscle preparation of the chick. Isolated biventer cervicis nerve-muscle preparation (Ginsburg and Warriner, 1960) was suspended in 20 ml of Krebs' solution, which was maintained at 37±0.5°C and bubbled with 95% O_2 and 5% CO_2. The preparation was stimulated indirectly with supramaximal rectangular pulses of 0.5 msec duration at a rate of 6 per minute. In some experiments the preparation was suspended in Locke's solution, containing NaCl, 9.0 g; KCl, 0.42 g; $CaCl_2$ 0.24 g; $NaHCO_3$, 0.5 g; and glucose 1.0 g per liter.

Phrenic nerve-diaphragm preparation of the rat. The techniques introduced by Bülbring (1946) was used. The preparations were suspended in 20 ml of Tyrode's solution which was kept at 37±0.5°C and oxygenated with 95% O_2 and 5% CO_2. The preparation was stimulated similarly as described for the biventer cervicis preparation.

Sartorius nerve muscle preparation of the frog. The sciatic nerve-sartorius muscle preparation was placed at room temperature (20-25°C) in 20 ml of aerated frog Ringer's solution. Indirect stimulation was applied similarly as described for the biventer cervicis muscle preparation. Direct stimulation was applied to the muscle after neuromuscular block, using pulses of 5 msec duration. The frog Ringer's solution contained, in grams per liter, NaCl, 6.5; KCl, 0.16; $CaCl_2$, 0.20; $NaHCO_3$, 0.5; Glucose, 1.0.

Rectus abdominis muscle preparation Isolated rectus abdominis muscle preparations were bathed in 20 ml of aerated frog Ringer's solution with or without calcium.

Isolated frog's heart The isolated frog's heart was prepared according to Straub's method.

Isolated rat atrial preparation The rat atrial preperation was prepared by the method described by Burn (1952) and suspended in a well oxygenated constant temperature bath (29°C) containing the Locke's solution, in which the amount of glucose was doubled (2 g/l). The contractions of the atria were recorded on a smoked drug.

Membrane Potentials For determination of membrane potentials, the conventional microelectrode recording technique (Fatt & Katz, 1951) was followed, using Grass P6 DC preamplifier and Tektronix 502A oscilloscope. The microelectrode was filled with 3M KCl and had 6 - 10 MΩ resistance. No capacity compensation for the microlectrode was incorporated. For the rat phrenic nerve-diaphragm preparation, Tyrode's solution, oxygenated with 95% O_2 + 5% CO_2, was used. The temperature was kept at 32 - 35 ± 0.5°C. For the frog nerve-sartorius muscle, the preparation was suspended in the Ringer solution, containing NaCl 117 mM, KCl 2.0 mM, $CaCl_2$ 1.8 mM and $NaHCO_3$ 6 mM, at the room temperature (20 - 24°C).

Terminal nerve spike Extracellular recording of the terminal nerve spike with a microelectrode having resistance of about 5 MΩ was performed on the frog sartorius muscle, according to the technique described by Hubbard & Schmidt (1963) and Katz & Miledi (1965). The muscle was immobilized

by adding 11 mM $MgCl_2$ to the Ringer solution. Under this condition, the terminal nerve spike potential could be recorded together with an EPP.

Twitch response of the guinea-pig ileum The method of Paton (1957) was used. Guinea-pig ileum was suspended in a bath containing 10 ml Krebs' solution at 30°C and stimulated co-axially with supramaximal rectangular pulses of 0.5 millisecond duration once every 10 seconds.

Electrocardiogram and blood pressure of the cat. Cats anesthetized with 60 mg/kg of chloralose were used. The electrocardiogram in lead II and the blood pressure of the right femoral artery were recorded with the polygraph, Grass Model 5. The Statham $P_{23}AC$ pressure transducer was used to record the blood pressure. The cardiotoxin was injected into the right femoral vein.

RESULTS

Zone electrophoretic separation As illustrated in Fig. 1, the venom migrated towards the cathode and separated into four fractions. They comprised approximately 14.3, 30.4, 10.2 and 29.5% of the extracted protein, respectively. The total protein recovery ranged from 58 to 70%. The cardiotoxic activity was located at Fraction IV on the basis of its effect on the frog's heart and the rat's atrium, while the neurotoxin located at Fraction II as described previously (Lee, 1963; Su, Chang, & Lee, 1966). The phospholipase A activity was found predominantly in Fraction I which had neither cardiotoxic nor neurotoxic activity. On the other hand, both the neurotoxic and cardiotoxic fractions exhibited only a very slight phospholipase A activity.

The cardiotoxic component, isolated by the fractional precipitation method as described by Sarker (1947), was further subjected to zone electrophoresis on starch and three fractions were obtained, corresponding to Fractions I, III and IV of the whole cobra venom (Fig. 2). Apparently Sarkar's cardiotoxin was not a single protein as pointed by Raudonat & Holler (1958).

Column chromatographic separation A typical chromatographic pattern is illustrated in Fig. 3. The crude venom was separated into 13 fractions. Fractions VII, VIII, X, XII and XIII are the main ones and they comprised approximately 3.0, 15.2, 12.9, 7.1 and 36.1% of the lyophilized eluates respectively. The total protein recovery was approximately 70% of original venom.

The first two fractions appear to be composed predominantly of nucleic acid since they had higher absorption at 260 mμ than at 280 mμ and showed little Folin-phenol reaction. Since column chromatography on CM-Sephadex given better separation than starch zone electrophoresis, experiments with the fractions isolated by the former procedure are described.

Toxicity in mice The LD50 and relative toxicity in mice of each chromatographic fraction are shown in Table 1. Among the toxic fractions, Fraction VIII, the neurotoxic fraction, is the most toxic and is 6 times more toxic than the whole venom; whereas Fraction XIII, the major cardiotoxic component, is only one third as toxic as the whole venom. The total recovery of toxicity is about 80%.

The toxic symptoms produced in mice by cardiotoxin included an initial stiffness of the limbs followed by spastic paralysis and then respiration was depressed. Most mice given lethal doses died within 4 hours although some of them died as late as 24 hours after envenomation. Before death, severe dyspnea was observed. The survival mice remained inactive for one or two days after the injection.

Effects on neuromuscular transmission Many of the chromatographic fractions were found to paralyse the skeletal muscle. Fractions V, VI, VII, VIII and IX comprise one group, which block the neuromuscular transmission without any direct effect on musculature as previously shown for cobra neurotoxin (Su, Chang & Lee, 1966; Chang and Lee, 1966). Fig. 4 shows the effect of Fraction VIII, which is the most toxic and identified to be the cobra neurotoxin, on biventer cervicis nerve-muscle preparation. The response to acetylcholine as well as to indirect nerve stimulation was blocked simultaneously without any contracture of the muscle on addition of the venom. For each fraction, its effect on neuromuscular transmission is paralleled to the toxicity tested in mice (Table 1).

In contrast, Fractions X, XII and XIII, comprize another group and at concentration of 10 μg/ml, induced a very marked contracture of biventer cervicis muscle (Fig. 5) and paralysis of the preparation followed only after the contracture (Fig. 6). The extent of contracture as well as the time required for neuromuscular block with these fractions is also parallel to the toxicity in mice (Table 1).

These fractions as typified by Fraction XIII are called "cardiotoxin" since they also have effect on heart as shown in the folllowing section. When these cardiotoxic fractions were added to a preparation immersed in calcium free Ringer's solution no contracture could be observed (Fig. 5) although the depolarizing effect of the cardiotoxin on the resting membrane (see below) persisted in this medium. On addition of calcium to the cardiotoxin-pretreated muscle, then contracture was induced.

These effects of cardiotoxin were further comfirmed in other preparations, such as rat phrenic nerve-diaphragm, frog sciatic nerve-sartorius and frog rectus abdominis muscle preparations though the toxin was slightly less active in these cases. The excitability of the preparation to indirect stimulation was usually depressed before the response to direct stimulation; the latter, however, was soon blocked on prolonged exposure to the toxin, indicating that both the musculature and nervous tissues were effected by cardiotoxin.

Effect on isolated frog heart High concentration of cardiotoxin, such as 100 μg/ml, produced ventricular arrest at systolic state within 20 minutes. The heart rate increased at first and then decreased afterwards. (Fig. 7) With lower concentration, such as 31.6 and 10 μg/ml, no cardiac arrest occurred but the rate of heart beat was accelerated similarly as with higher concentrations; with still lower concentration such as 1 μg/ml, only augmentation of systole was observed.

Effect on the rat's atrial preparation When 1 to 5 μg/ml of the cardiotoxin was applied to the atrial preparation, a transient slight positive inotropic

effect followed by negative inotropic effect with gradual decrease of atrial rate was observed; thereafter, the atrium ceased to beat within 20 minutes (Fig. 8). When the time needed to arrest the atrial contraction at various concentration of cardiotoxin was compared with that of whole venom, cardiotoxin appeares to be slightly more "cardiotoxic" than the whole venom.

Effect on resting membrane potential The muscle fibres of either the rat diaphragm or frog sartorius was inserted at random with microelectrodes at both endplate and non-endplate zone and the resting membrane potentials recorded. As shown in Table 2, cardiotoxin as well as the whole venom but not neurotoxin markedly depolarized both the diaphragm and sarotrius muscles. The effect of cardiotoxin appeared to be potentiated by phospholipase A pretreatment. Elimination of calcium from the medium did not protect the muscle from depolarization.

It appeares that the non-specific contracture-inducing effect of cardiotoxin may be explained on the basis of membrane depolarization. In a preliminary experiment rat atrium was like-wise depolarized by this toxin.

Effect on nerve terminal spikes To see whether the nervous element is also effected by cardiotoxin, nerve terminal spikes was recorded with endplate potentials with an extracellular microelectrode in frog sartorius nerve-muscle preparations according to the method described by Katz & Miledi (1965). Fig. 9 shows that on addition of 10 μg/ml of cardiotoxin the end-plate potential was rapidly abolished as the membrane potential decreased. Subsequently, the

nerve terminal spike also disppeared on prolonged exposure to the cardiotoxin, a direct evidence that cardiotoxin disturbes the conduction of impulses in the nerve axon.

Effect on guinea-pig ileum. Cardiotoxin at concentration as low as 1 μg/ml, produced a marked contraction of the guinea-pig ileum following a latent period of about 15 to 30 sec. (Fig.10). The contracture was transient and the muscle tone usually falled to the normal level after about 5 min. even in the presence of the toxin. As in the skeletal muscle elimination of calcium from the Tyrode solution markedly reduced othe contracture. There was a remarkable tendency of tachyphylaxis in the cardiotoxin-induced contraction so that the response of the ileum to cardiotoxin reduced considerably after several times of application of cardiotoxin (Fig. 10). The development of tachyphylaxis could not be prevented by repeated washing for prolonged time up to 30 min.

Antagonism to the stimulant action of cardiotoxin. Pyribenzamine (0. 2 μg/ml), which completely blocked histamine response, did not affect the stimulant action of cardiotoxin on the gut. Hexamethonium (10 μg/ml) or mecamylamine (5 μg/ml) also failed to antagonize the response of the gut to cardiotoxin. However, as shown in Fig. 11, the response produced by cardiotoxin was greatly reduced by atropine (0. 05 μg/ml). The combination of morphine (1 μg/ml) and phenoxybenzamine (0. 05 μg/ml), which blocked the responses to 5-hydroxytryptamine and histamine and reduced those to acetylcholine, also partially inhibited the stimulant effect of cardiotoxin.

Effect on the response to pharmacological agonists. In addition to the stimulant effect of cardiotoxin on the guinea-pig ileum, it was found that the responses to various smooth muscle stimulants were also affected by cardiotoxin. The motor response to nicotine (0.6 to 1.4 μg/ml) was first enhanced but then depressed 5 to 10 min. after addition of 10 μg/ml of cardiotxin (Fig. 12). The response to 5-hydroxytryptamine (0.4 to 0.7 μg/ml), on the other hand, was considerably reduced on addition of cardiotoxin (1 to 10 μg/ml) without any initial potentiation. The responses to histamine or acetylcholine were also decreased by cardiotoxin (10 μg/ml) but to a less extent in comparison with 5-hydroxytryptamine.

When exposed to high concentrations of cardiotoxin (40 μg/ml), all of the responses to nicotine, 5-HT, histamine and acetylcholine were almost completely inhibited and no recovery occurred upon washing.

Effect on twitch response of the guinea-pig ileum stimulated coaxially. At submaximal stimulus strength, the twitch response of the ileum to coaxial stimulation was potentiated by cardiotoxin at concentrations from 1 to 10 μg/ml. The potentiation of twitch by cardiotoxin attained its maximum in 2 to 3 min., lasted about 10 min. and then followed by progressive depression. (Fig. 13). The time-course of the effect of cardiotoxin on the twitch response induced by co-axial stimulation, therefore, corresponds to that of the effect on the response to nicotine. On the other hand, when stimulated supramaximally no potentiation was observed. High concentrations of cardiotoxin (50 to 100 μg/ml) completely abolished the response and direct electrical stimulation

with 100 to 150 V, 5 msec. duration, also failed to cause any response on the paralysed preparation (Fig. 14).

Action on electrocardiogram and blood pressure of cats. The intravenous injection of the cardiotoxin in a dose of 0.1 mg per kg body weight caused no significant changes on ECG except decrease of heart rate. When the dose was raised to 0.5 mg per kg body weight, the following changes in ECG were observed (Fig. 15): P-R interval was prolonged, T wave became inverted and ST segment depressed, while Q-T interval and QRS complex were unaffected. Frequent ventricular premature contraction and trigeminal rhythm also occurred within 2 to 5 minutes after the injection. These effects reached maximum 10 min after the administration of cardiotoxin. Blood pressure decreased markedly. The abnormal findings disappeared and the blood pressure recovered about 50 minutes after the injection. After injection of 1 mg per kg, the changes of ECG were more marked and irreversible. Complete A-V block with aberrant QRS-T complex and idioventricular rhythm were observed. QT interval increased slightly. Systolic pressure decreased much more than diastolic pressure and finally fell to nil within 2 to 20 minutes. (Fig. 15).

DISCUSSION

Although "neurotoxin" is the major toxic component of cobra venom by virture of its peripheral respiratory paralytic action in many animals, cats as well as other animals, which were envenomed with cobra venom and maintained by artificial respiration, would finally die of cardiovascular failure. This indicates that some other components acting on the cardiovascular system also contribute to the toxicity of cobra venom. One component was isolated by Sarker (1947) and named "cardiotoxin" though it still contained many other components when tested by electrophoresis, and its pharmacological actions remained obscure.

The action of the cardiotoxin on isolated frog's heart resembles that of digitalis in some way. There was some increase in contraction height on addition of cardiotoxin and, at higher doses, systolic arrest occurred. It has been, therefore, suggested that cardiotoxin has digitalis -like action. However, in the rat atrium the inotropic effect was of very short duration and was soon followed by complete suppresion of the contraction. Electrocardiographic findings show that cardiotoxin causes depression of ST segment, inversion of T wave, prolongation of P-R interval and A-V block as digitalis does. These findings, however, do not necessarily mean that cardiotoxin acts like digitalis since the most basic effect of digitalis, enhancement of contractility, is not reflected in the electrocardiograph. Moreover, cardiotoxin increased the Q-T interval in stead of shortening, a characteristic of digitalis action. In fact, there is one basic difference between cardiotoxin and digitalis; i. e.,

depolarization of the membrane by the fomer.

Experiments on neuromuscular preparations have revealed that skeletal muscles are as sensitivie as heart muscle to cardiotoxin. All of the tested preparations responded to cardiotoxin with a marked contracture and with a marked reduction in the membrane potentials at both end-plate and non-end-plate zone. Since the contracture of muscles needs calcium it is likely that the depolarization of the cell membrane is the primary action of cardiotoxin. It may be infered further that the cardiotoxic effect also may be a result of depolarization of the heart muscle. Therefore, cardiotoxin appears to be a rather general poison to cell membranes. Failure in the conduction within nerve axon or in the ganglionic transmission induced by cardiotoxin are evidences for this suggestion.

Experiments using guinea-pig ileum show that the action of cardiotoxin extends to smooth muscles. In addition to its direct stimulant effect on the ileum, the response of the muscle to coaxial stimulation, and to application of acetylcholine, histamine, 5-hydroxytryptamine, and barium were suppressed. These evidences again indicate that cardiotoxin acts on a common site to all of these agents, cell membrane. Transient initial potentiation of the muscle response to nicotin and to submaximal electrical stimulation suggests that the nervous elements of the ileum are also involved as those of skeletal muscles and ganglia.

It may be concluded from the evidences of present experiments that cardiotoxin has general effect on cell membrane with depolarization and consequently impairs the functions associated with cell membrane.

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Table 1. Toxicity in mice protein recovery and time required to induce N-M blockade in biventer cervicis muscle of the CM-Sephadex fractionated venom.

Fr. no.	LD_{50} μg/g	Potency ratio	N-M block 1×10^{-5} min	Protein recovery %
I	100	0.0044	No N-M block	7.6
II				0.84
III				0.72
IV				3.6
V	0.18	2.4	8	1.7
VI	0.44	1.0	8	2.6
VII	0.68	0.67	13	8.0
VIII	0.074	6.0	6	15.2
IX	5.6	0.08	19	1.7
X	3.0	0.15	20*§	12.9
XI	100	0.0044	No N-M block	1.7
XII	4.3	0.10	23*§	7.1
XIII	1.48	0.30	10*§	36.1
CV	0.44	1.0	8*§	

*: Contracture occurred in the biventer cervicis muscle.

§: with cardiotoxic action

Table 2. Effects on resting membrane potentials of crude cobra venom and isolated components.

Membrane potentials (mV±S. D.) were recorded from both endplate and non-endplate zone of muscle fibres at the indicated periods after addition of 10 μg/ml of each agent. n = number of observations.

	Control	0-5 min.	5-10 min.	10-15 min.	15-20 min.
Rat diaphragm					
Crude venom	83.0±3.7 (n=30)	49.3±8.3 (n=9)	34.0±17.2 (n=11)	29.0±6.2 (n=9)	23.0±6.8 (n=8)
Neurotoxin	78.1±4.4 (n=28)	81.4±4.4 (n=11)	77.0±3.9 (n=5)	78.4±4.2 (n=10)	76.0±4.8 (n=7)
Cardiotoxin	81.4±4.1 (n=30)	73.7±8.5 (n=11)	54.4±9.7 (n=10)	45.3±14.2 (n=9)	28.9±12.8 (n=12)
Phospholipase A	70.2±6.5 (n=20)	67.4±2.8) (n=3)	69.8±5.9 (n=12)	-	69.7±5.4 (n=22)
Phospholipase A + Cardiotoxin	66.0±8.1 (n=26)	34.5±9.9 (n=12)	27.6±12,3 (n=18)	18.9±10.4 (n=22)	13.7±3.5 (n=4)
Frog sartorius					
Crude venom	92.9±5.4 (n=23)	78.9±14.2 (n=10)	54.2±26.3 (n=12)	29.2±12.2 (n=13)	22.8±11.3 (n=17)
Neurotoxin	92.0±2.6 (n=33)	88.3±5.5 (n=11)	87.0±4.9 (n=10)	85.6±5.3 (n=10)	86.9±6.5 (n=11)
Cardiotoxin	88.6±1.2 (n=22)	70.9±15.1 (n=13)	36.0±3.2 (n=18)	23.8±7.0 (n=13)	17.0±2.7 (n=9)

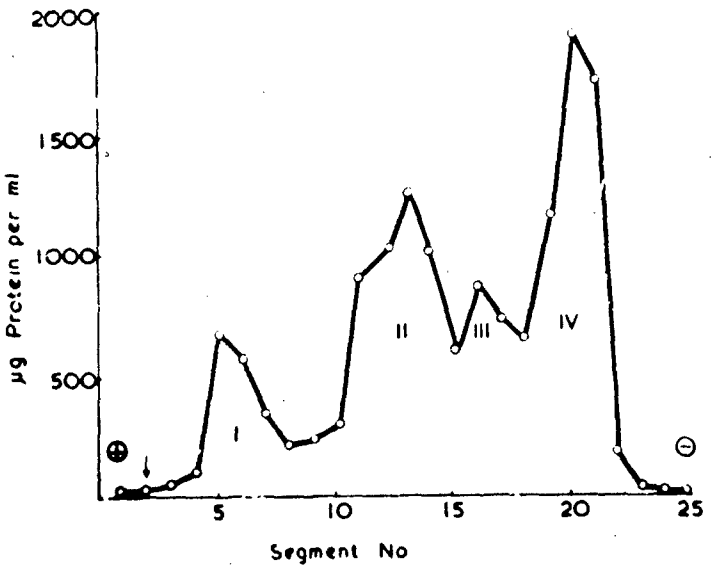


Fig. 1. One hundred mg of cobra venom was charged at segment No. 2, indicated by an arrow in the figure, of potato starch packed into a semicylindrical glass trough, 40 x 4 cm. Acetate buffer of pH 5.0, ionic strength 0.05 plus sodium chloride, ionic strength 0.05, was used. An average potential difference of 180 V was applied between the two ends of the trough for 24 hrs at 4°C.

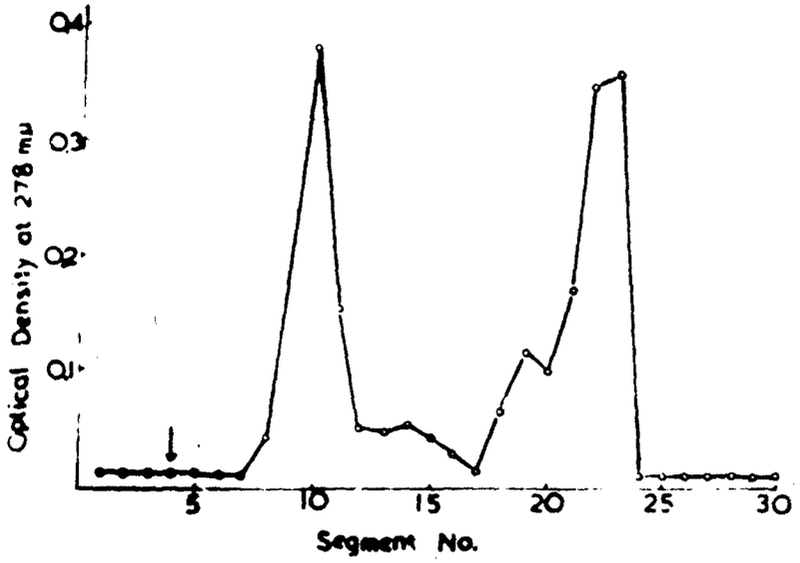


Fig. 2. Zone electrophoresis of the substance isolated from the venom of Formosan cobra by Sarkar's method. 20 mg of this substance was charged at segment No. 4 under the same conditions as Fig. 1.

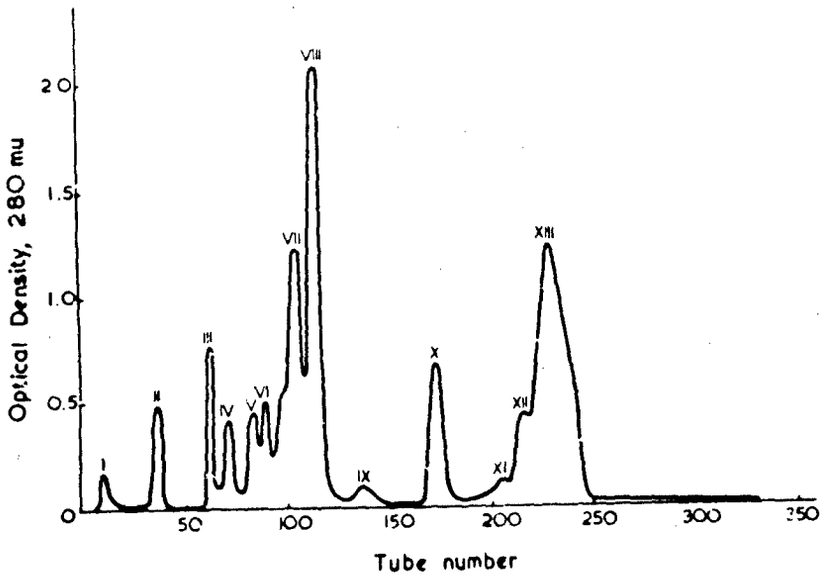


Fig. 3. Fractionation with CM-Sephadex. Venom charged was 350 mg and eluted with gradient ammoniumacetate buffer increment, from 0.005 M, pH, 5.0 to 0.9 M, pH. 7.0.

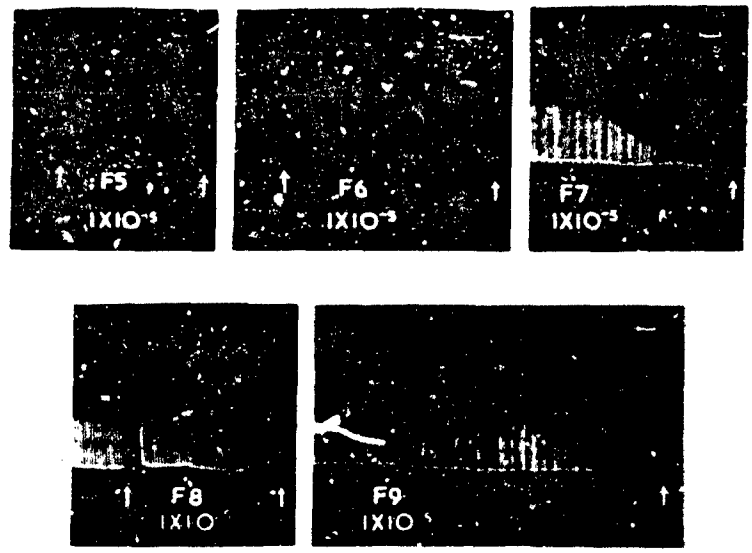


Fig. 4. Effects of neurotoxic fractions on the biventer cervicis nerve-muscle preparations of the chick. Indirect stimulation once every 10 sec. was applied. At arrows stimulation was stopped and ACh, 2×10^{-5}, was added and then washed after 30 sec.

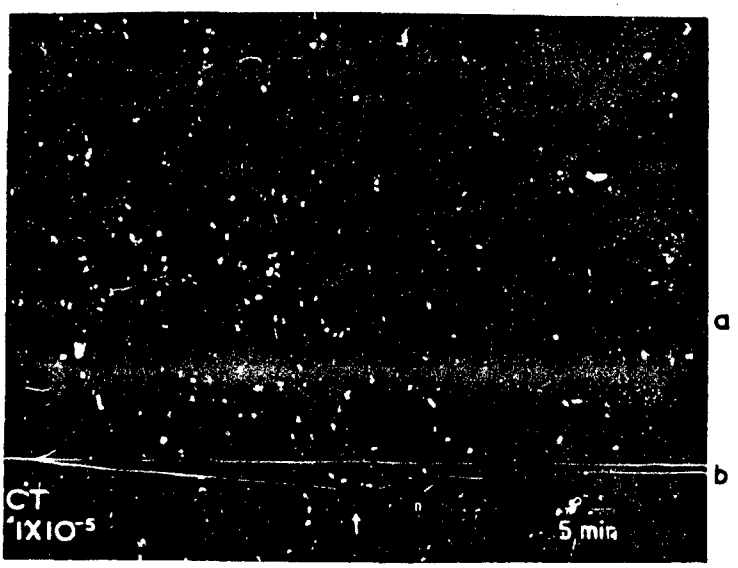
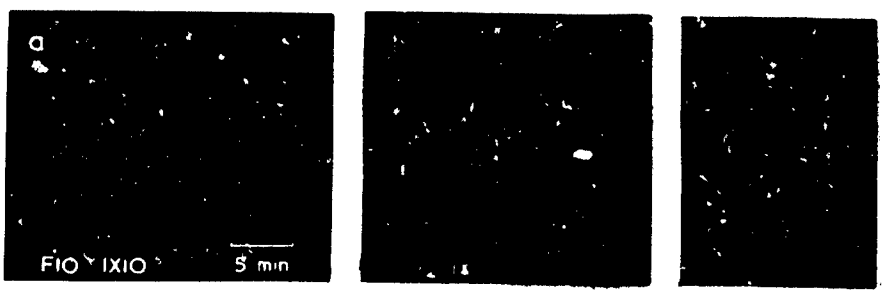


Fig. 5. Effects of cardiotoxin on the chick's biventer cervicis muscle a: normal Ringer's solution. b: Ca-free Ringer's solution, switched to normal Ringer's solution at arrow indicated.



CHICK BIVENTER CERVICIS

Fig. 6. Effects of cardiotoxic fractions on the biventer cervicis muscles, under the same conditions as in Fig. 4.

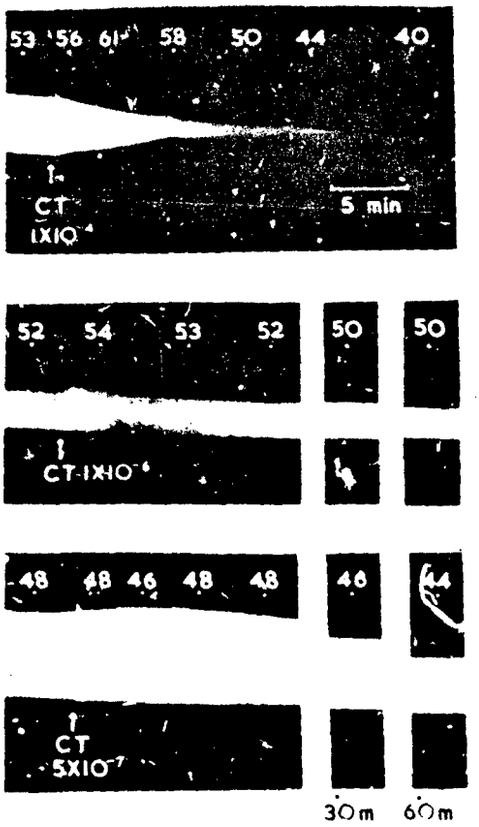


Fig. 7. Effects of various concentrations of cardiotoxin on isolated frog hearts. Figures indicated show heart rates.

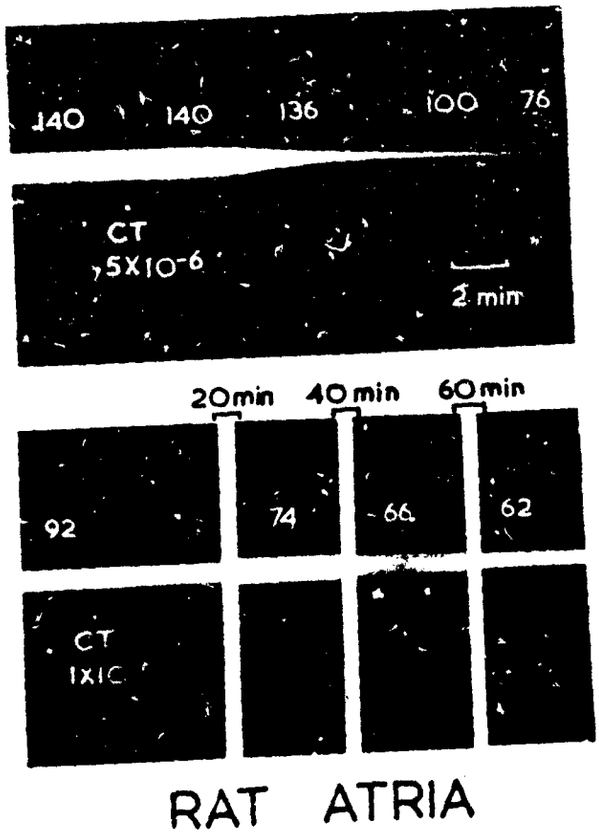


Fig. 8. Effects of cardiotoxin on the isolated rat atria.

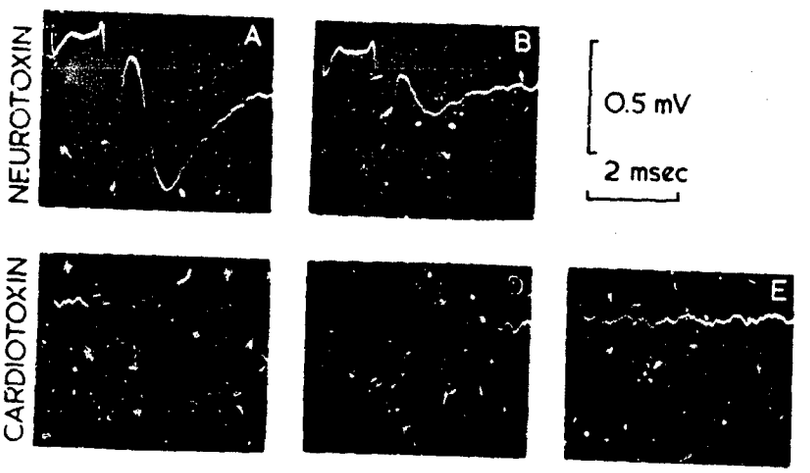


Fig. 9. Effects of cobra neurotoxin and cardiotoxin on terminal nerve spikes and EPPs.

Frog nerve sartorius preparations immobilized by 11 mM $MgCl_2$.

A and C: Control terminal nerve spike and EPP.

B: 15 min. after addition of 10 μg/ml of cobra neurotoxin.

D and E: 10 and 50 min. after addition of 10 μg/ml of cardiotoxin respectively.

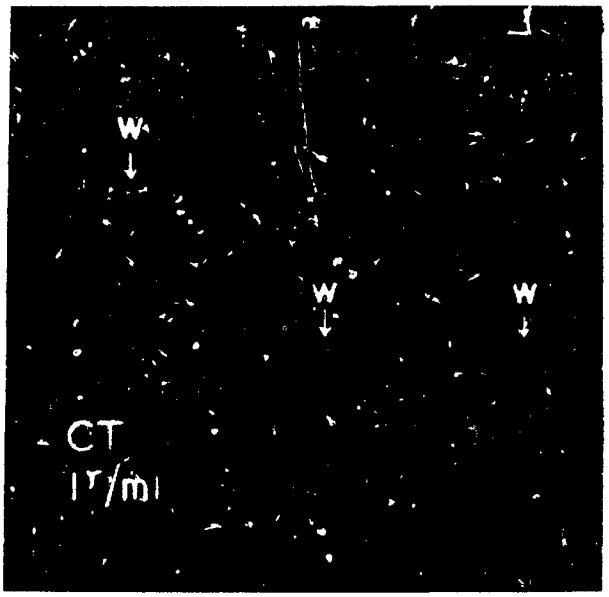


Fig. 10. Effect of cardiotoxin on isolated guinea-pig ileum. Interval between doses of cardiotoxin (1 μg/ml) was 15 min. Arrows indicate washings.

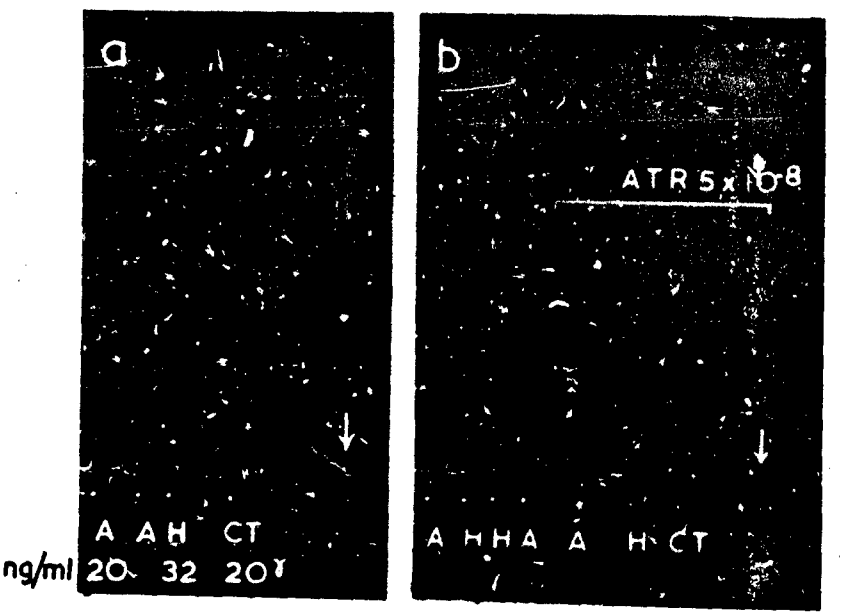


Fig. 11. Inhibition of contraction due to cardiotoxin by atropine on the guinea-pig isolated ileum. Two adjacent segments of mid-ileum were used in the experiment and their responses to acetylcholine, histamine and cardiotoxin were recorded. Ileal segment (b) was treated with atropine and was left in contact with the drug for the duration indicated by the bracket above the trace. In ileal segment (a) atropin was not added. Arrows indicate washings; A, acetylcholine 20 ng/ml; H, histamine 32 ng/ml; CT, cardiotoxin 20 μg/ml; ATR, atropine 50 ng/ml.

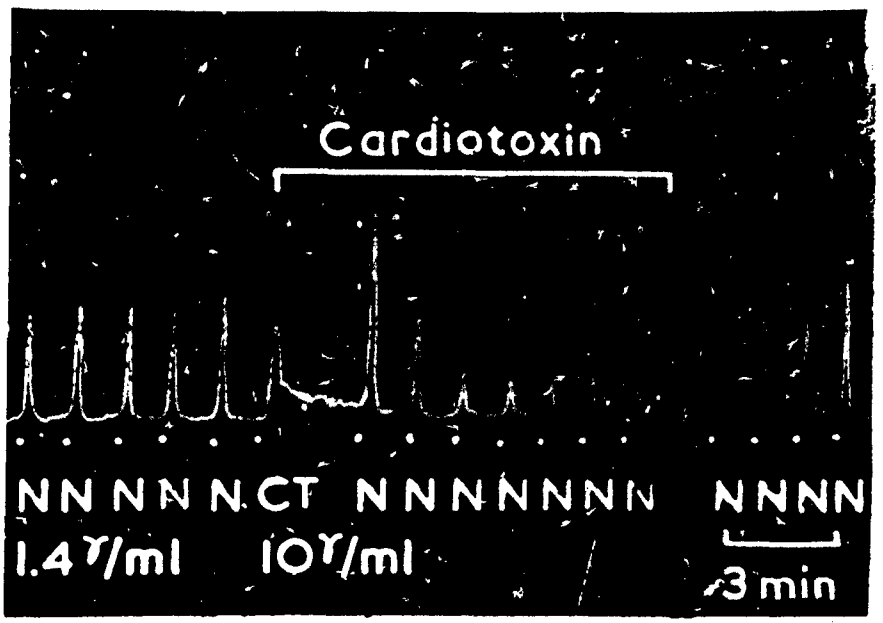


Fig. 12. Effect of cardiotoxin on the response of the ileum to nicotine. Addition of cardiotoxin (10 μg/ml) is indicated by the bracket in the graph, N; nicotine 1.4 μg/ml;

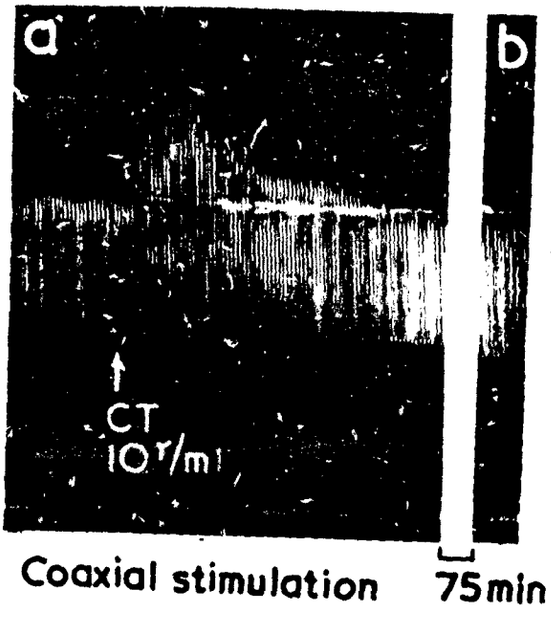


Fig. 13. Guinea-pig ileum preparation stimulated co-axially at submaximal strength, with frequency 0.1/sec, duration 0.5 msec. At the arrow cardiotoxin 1 x 10^{-5} g/ml was added. b: 90 min. after addition of cardiotoxin.

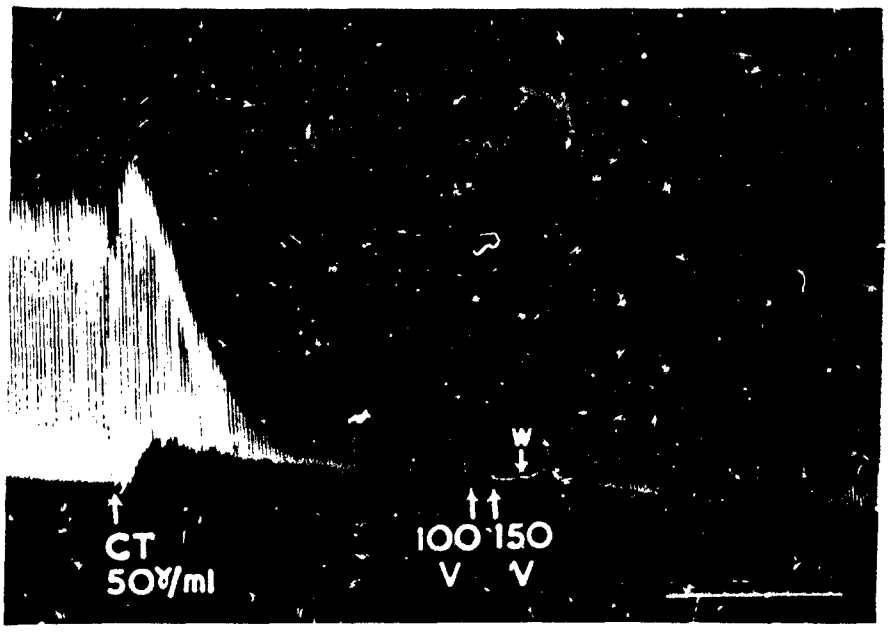


Fig. 14. Guinea-pig ileum preparation stimulated co-axially at supramaximal strength. At the arrow cardiotoxin 5 x 10^{-5} g/ml was added. a and b were responses to single shocks of 100 V and 150 V respectivoly (duration 5 msec). w: washing.

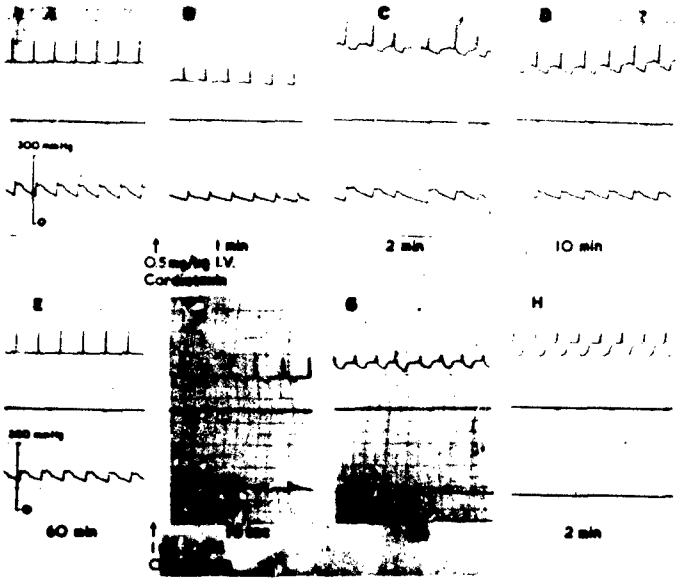


Fig. 15. Action of the cardiotoxin on electrocardiograms and blood pressure of the cat. Cat, 2 Kg, chloralose 60 mg/kg. Upper-middle-lower tracings trac ings show ECG (lead II), time interval (per second) and blood pressure (mm-Hg) respectively. A. Control; B. 1 min. after injection of 0.5 mg/kg cardiotoxin; C. 2 min. after the injection; D 10 min. after the injection; E. 60 min. after the injection; F. immediately after injection of 1 mg/kg cardiotoxin into the same cat. G. 1 min. after the injection; H. 2 min. after the injection.