Mem. Inst. Butantan
Simp. Internac.
33(3):993-1000, 1966

45. PHARMACOLOGY OF CRYSTALLINE CROTOXIN. III. CARDIOVASCULAR AND RESPIRATORY EFFECTS OF CROTOXIN AND *CROTALUS DURISSUS TERRIFICUS* VENOM.

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The venom of *C. d. terrificus* besides producing skeletal muscle paralysis and renal insufficiency, gives rise to acute disturbances of blood pressure and respiration as it was demonstrated by Arthus (1) and others (2, 3). The main purpose of the present study was to investigate the actions of crotoxin on blood pressure and respiratory function so that the role it represents in the genesis of the disturbances evoked by the venom could be evaluated. Experiments with the venom were also done for adequately evidencing the differences of action and potency between crotoxin and venom.

The mechanism through which acute respiratory disturbances are caused by the venom did not seem to be clear. Houssay (4) concluded from experiments carried out on the perfused and isolated head preparation of dogs that the venom of *C. d. terrificus* exerts direct actions on the central nervous system. He verified that signs of respiratory stimulation and depression appeared in the isolated head following the injection of the venom in the perfuser dog. However, the experiments of Houssay do not eliminate the possibility that the respiratory disturbances could be also reflexly generated through stimulation of chemoreceptors at the carotid and aortic bodies, or of receptors in the lungs. Therefore some experiments were planned to investigate this point. The results suggest that the direct action on the medulla is perhaps less important in causing the acute respiratory effects than the reflex ones.

Material and methods

The venom of *C. d. terrificus* used in this research was extracted as previously reported (5) from snakes captured in Goiás; it belonged to the variety devoid of crotamine. The batches of crotoxin utilized in the experiments were crystalline preparations obtained according to procedures already referred to in the previous paper (5).

This study was supported by a grant from the "Fundação de Amparo à Pesquisa do Estado de São Paulo".

The acute effects evoked by crotoxin and the venom on blood pressure and respiration were compared in dogs anaesthetized with sodium pentobarbital (30 mg/Kg, i.v.). The effect they produced on the haematocrit value was also investigated in most of the experiments. The dogs were prepared for recording arterial blood pressure and respiration as it was described in a previous work (6).

Samples of blood were withdrawn for the determination of the haematocrit values 30 minutes and 1, 2 and 3 hours after the injection of the venom or crotoxin. Twenty-four dogs were employed in the experiments: sixteen animals were injected with crotoxin and eight with the venom. Crotoxin from two batches (crotoxin n.º 5 and n.º 14) was used. No haematocrit value determinations were made in the experiments, in numbers of eight, in which crotoxin n.º 14 was employed. The dose of 0.25 mg/Kg of crotoxin or of the venom was used in all experiments. The arterial blood pressure and respiration were usually recorded for 3 hours after administration of the venom or crotoxin.

The effects produced by crotoxin and the venom were also compared by injecting them in the same dog. Two or three doses of 0.25 mg/Kg, conveniently spaced, of crotoxin were administered in order that the animal become irresponsive to its hypotensive action. Then 0.25 mg/Kg of the venom were injected.

The experiments planned to investigate the mechanisms involved in the genesis of the respiratory effects caused by the venom were carried out in four dogs with both vagi cut, and in ten whose carotid sinus nerves, in addition, were sectioned. Occlusion for 30 or 45 seconds of the common carotid arteries was made before the injection of the venom to test the denervation of the carotid sinus regions. The dogs were anaesthetized with sodium pentobarbital. The dose of 0.25 mg/Kg of the venom was also employed in these experiments.

Results

Table I shows the percentages of the maximums of blood pressure falls observed in the experiments in which crotoxin n.º 5 and crotoxin n.º 14 were used, and their means $32 \pm 6.5\%$ and $25 \pm 6.44\%$. The difference between these means is not statistically significant ($P > .05$). Both of them, however, are significantly smaller ($P < .01$) than the mean $58.3 \pm 5.6\%$, obtained from the experiments with the venom *C. d. terrificus* (Table II). The venom was also much more potent in increasing the haematocrit value. Table I and II show the percentages of the increases which were demonstrated 30 minutes after the injection of crotoxin n.º 5 and the venom, as well as their means $20.2 \pm 4.4\%$ and $43.5 \pm 5.9\%$. The difference between these means are highly significant ($P << .01$).

The hypotensive effect elicited by crotoxin was always reversible; the arterial blood pressure usually attained its primitive level in less than two hours after its administration. This did not always happen in the experiments with the venom: In two out of eight dogs, the effect on blood pressure was irreversible and the animals died from the hypotension in less than one hour. However, the blood pressure of the other dogs recovered within 2 or 3 hours. The effects evoked by the venom and crotoxin on blood pressure were also qualitatively different from each other (Figs. 1, 2).

TABLE I — MAXIMUMS OF BLOOD PRESSURE DEPRESSIONS AND INCREASES IN THE HEMATOCRIT VALUES CAUSED BY THE INTRAVENOUS ADMINISTRATION OF 0.25 MG/KG OF CROTOXIN

Crotoxin no. 5 *			Crotoxin no. 14 **	
Experiment (no.)	Maximum fall of (%)	Increase in the hematocrit value (%)	Experiment (no.)	Maximum fall of blood pressure (%)
1	40.9	7.5	1	29
2	30.6	17.0	2	15
3	56.2	40.0	3	35
4	37.7	17.0	4	0
5	19.3	12.0	5	10
6	14.2	17.8	6	50
7	55.5	39.4	7	49
8	6.5	10.6	8	17
	Mean ± S.E.	Mean ± S.E.		Mean ± S.E.
	32.6 ± 6.5%	20.2 ± 4.4%		25.6 ± 6.44%

* LD_{50} for mice (i.v.) 82 (66.9 to 101.4) mcg/Kg.
** LD_{50} for mice (i.v.) 80.3 (60.8 to 108.9) mcg/Kg.

TABLE II — MAXIMUMS OF BLOOD PRESSURE DEPRESSIONS AND INCREASES IN THE HEMATOCRIT VALUES CAUSED BY THE INTRAVENOUS ADMINISTRATION OF 0.25 MG/KG OF THE VENOM OF *C. D. TERRIFICUS*

Experiment (no.)	Maximum fall of blood pressure (%)	Increase in the hematocrit value (%)
1	37.7	44.7
2	71.7	50.0
3	70.0	44.2
4	73.3	21.5
5	50.7	35.1
6	60.2	56.4
7	69.7	71.7
8	33.3	24.3
	Mean ± S.E.	Mean ± S.E.
	58.3 ± 5.6%	43.5 ± 5.9%

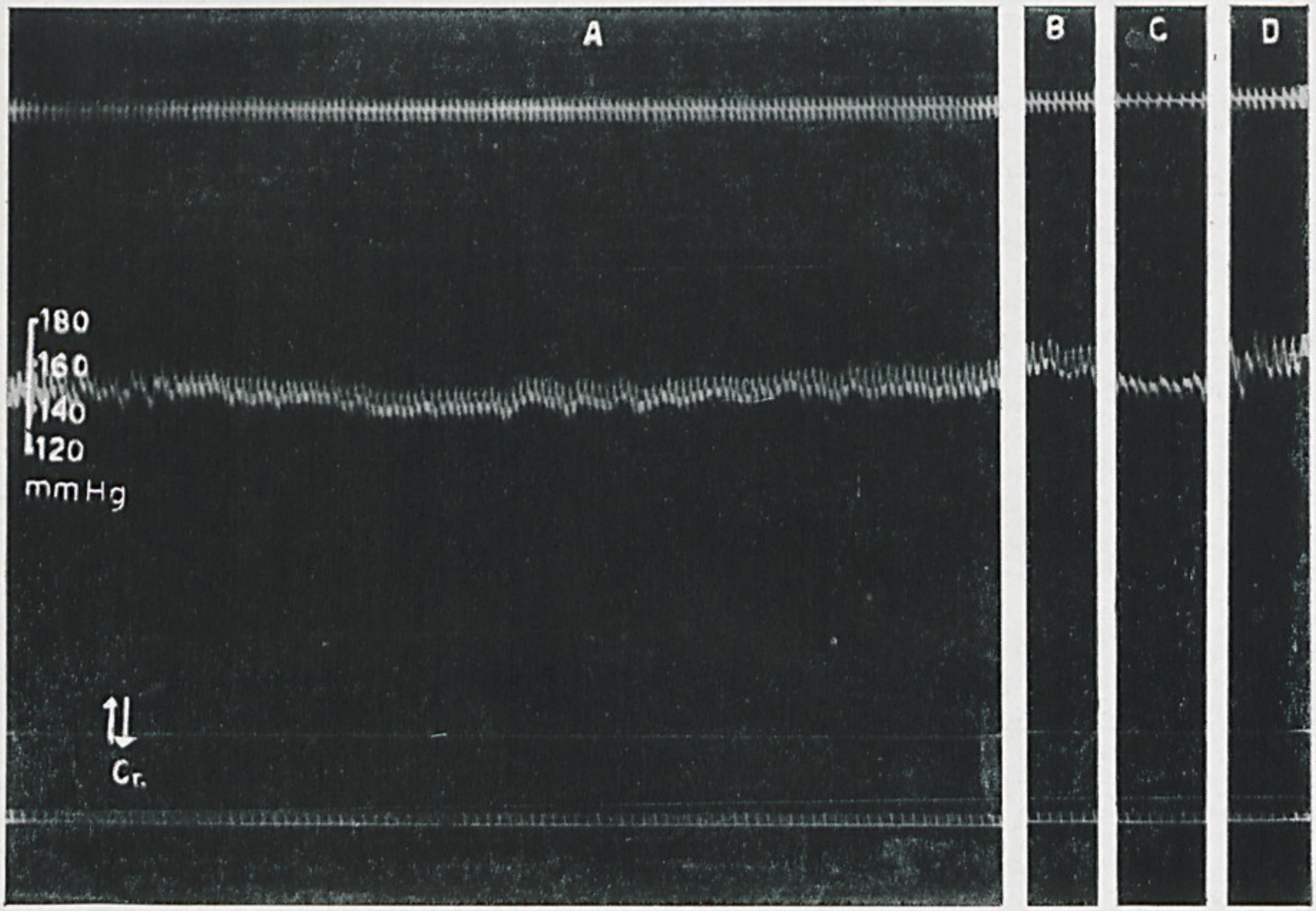

Fig. 1 — Dog, 10.5 Kg, sodium pentobarbital. Records of respiration and blood pressure. At Cr., 0.25 mg/Kg of crotoxin were injected. At B, C and D, records taken 30, 60 and 90 minutes after A. Time 6/6 seconds.

Crotoxin produced a rather gradual blood pressure fall which began to appear from 2 to 3 minutes after its administration. After the venom, on the other hand, an abrupt and transient fall of blood pressure appeared within the first 30 seconds and was followed by a pressor effect of one or two minutes duration. Thereafter a less abrupt and prolonged fall occurred in all experiments. It was usually more intense than the first fall.

Respiration did not seem to be acutely modified by crotoxin (Fig. 1). The venom, on the other hand, caused an intense and transient increase in the frequency and amplitude of the respiratory movements a few seconds after its administration. This was followed by apnoea of brief duration and then by tachypnoea which persisted for more than one hour (Fig. 2).

Haemolysis was demonstrated in all experiments in the blood extracted two hours after the injection of crotoxin or venom.

The striking differences in the effects elicited by crotoxin and venom were also revealed by injecting them in the same dog. The venom caused the usual disturbances on blood pressure (and also on respiration) after the dog was made irresponsive (tachyphylaxis) to the hypotensive action of crotoxin. Fig. 3 shows the records of an experiment in which the blood pressure was not altered by crotoxin. Nevertheless, the venom produced, as usually, the characteristic effects on blood pressure and respiration.

The apnoea usually elicited by the venom did not occur after its administration in the dogs whose vagi had been cut. In its place, a brief phase of decreased respiratory excursions was observed (Fig. 4). The other acute respiratory disturbances evoked by the venom were definitely attenuated in all experiments. In eight out of the ten dogs whose vagi and carotid sinus nerves had been sectioned, the venom gave rise to only a slight increase in the respiratory frequency and to a very brief phase in which a small diminution in the respiratory amplitude occurred. In two of these animals the respiratory effects were almost suppressed (Fig. 5). On the other hand, in two other experiments the respiratory disturbances were nearly as intense as those observed in dogs with the vagi and carotid sinus nerves intact. However, the apnoea did not occur.

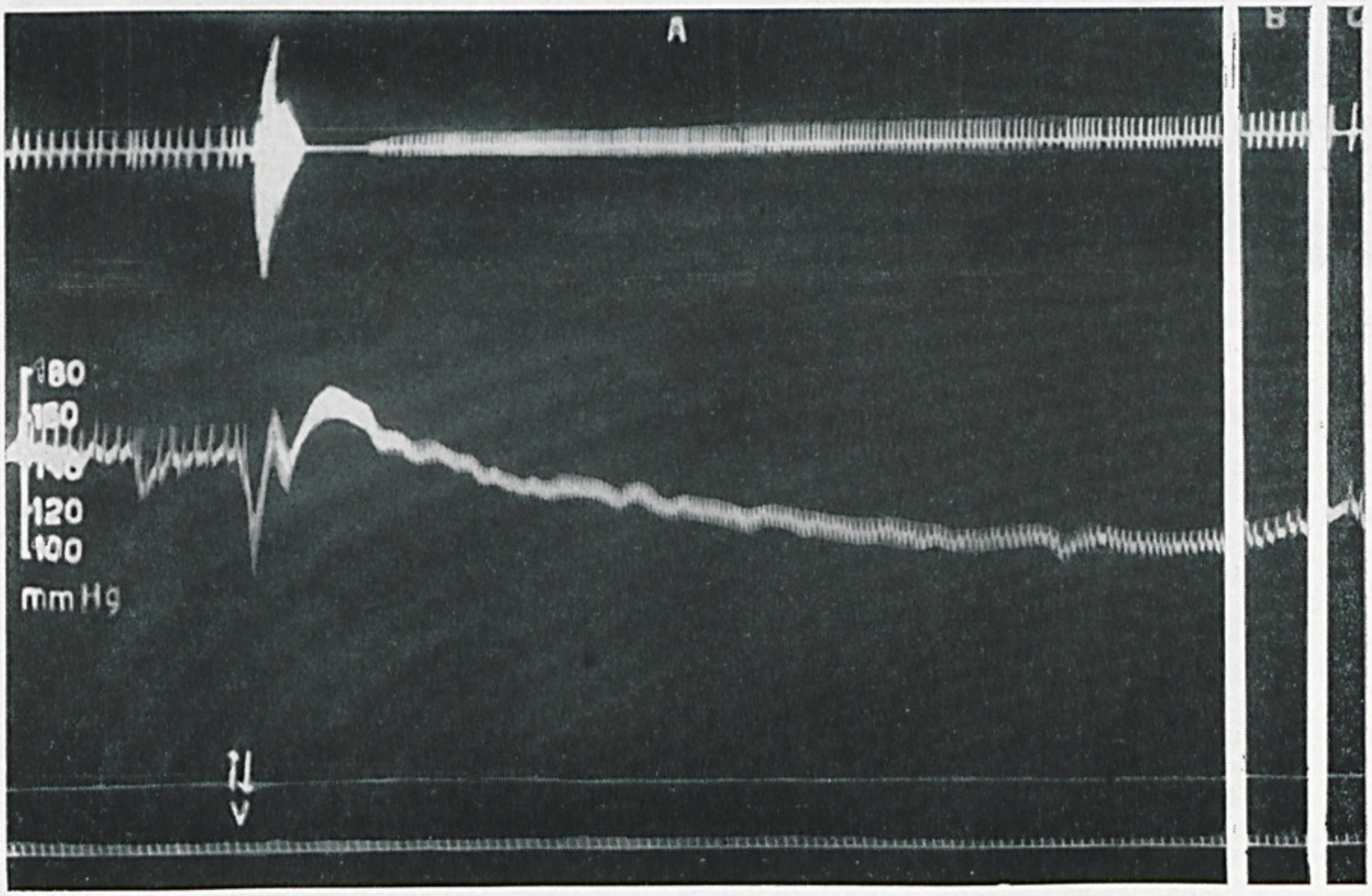

Fig. 2 — Dog, 7 Kg, sodium pentobarbital. Records of respiration and blood pressure. At V., 0.25 mg/Kg of *C. d. terrificus* venom were injected. At B, C and D, records taken 30, 60 and 150 minutes after A. Times 6/6 seconds.

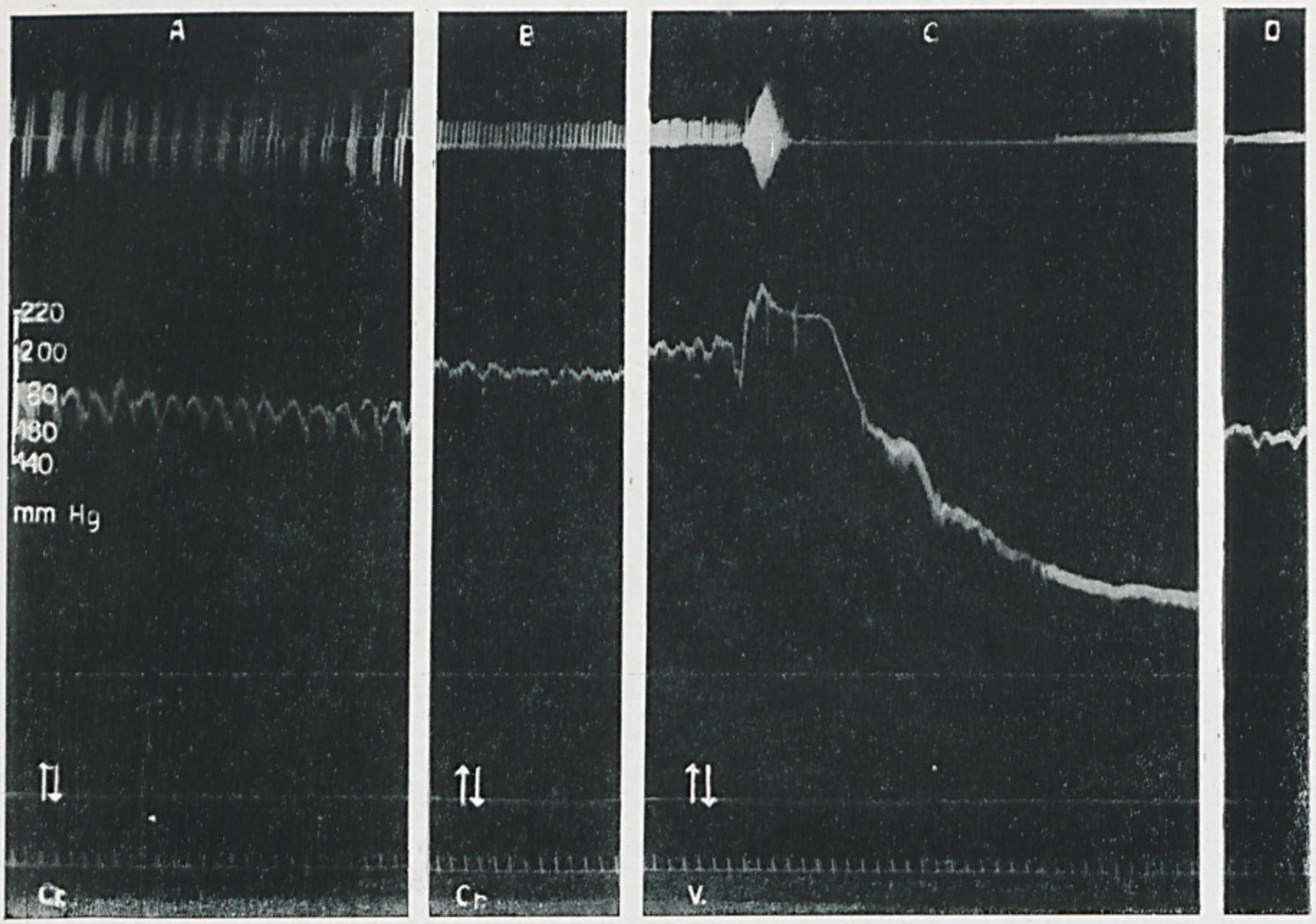

Fig. 3 — Dog, 10 Kg, sodium pentobarbital. Records of respiration and blood pressure. At Cr. (panel A and B), 0.25 mg/Kg of crotoxin were injected; at V. (panel C), 0.25 mg/Kg of the venom of *C. d. terrificus* were administered. At B, C and D, records taken 1, 2 and 3 hours after A. Time 6/6 seconds.

Discussion

The results of the present investigation show that the remarkable acute disturbances provoked by the venom of the South American rattlesnake on circulation and respiration are not due to crotoxin. Neither can they be attributed to crotamine because the venom employed in the experiments did not contain that toxin. Therefore, it can be inferred that, besides these, there are other components of the venom of *C. d. terrificus* whose pharmacological actions must play an important role in clinical envenomation, especially in the genesis of shock, a condition frequently exhibited by the patients bitten by that snake (7). The separation as well as the pharmacological and immunological study of such components is highly desirable.

It is surprising that, being endowed with phospholipase A activity, crotoxin exhibits so small hypotensive and shocking potencies. Lysolecithin, in effect, besides being hemolytic, produces in dogs and cats an abrupt fall of blood pressure and releases histamine (8). Feldberg, Holden and Kellaway (8) attributed

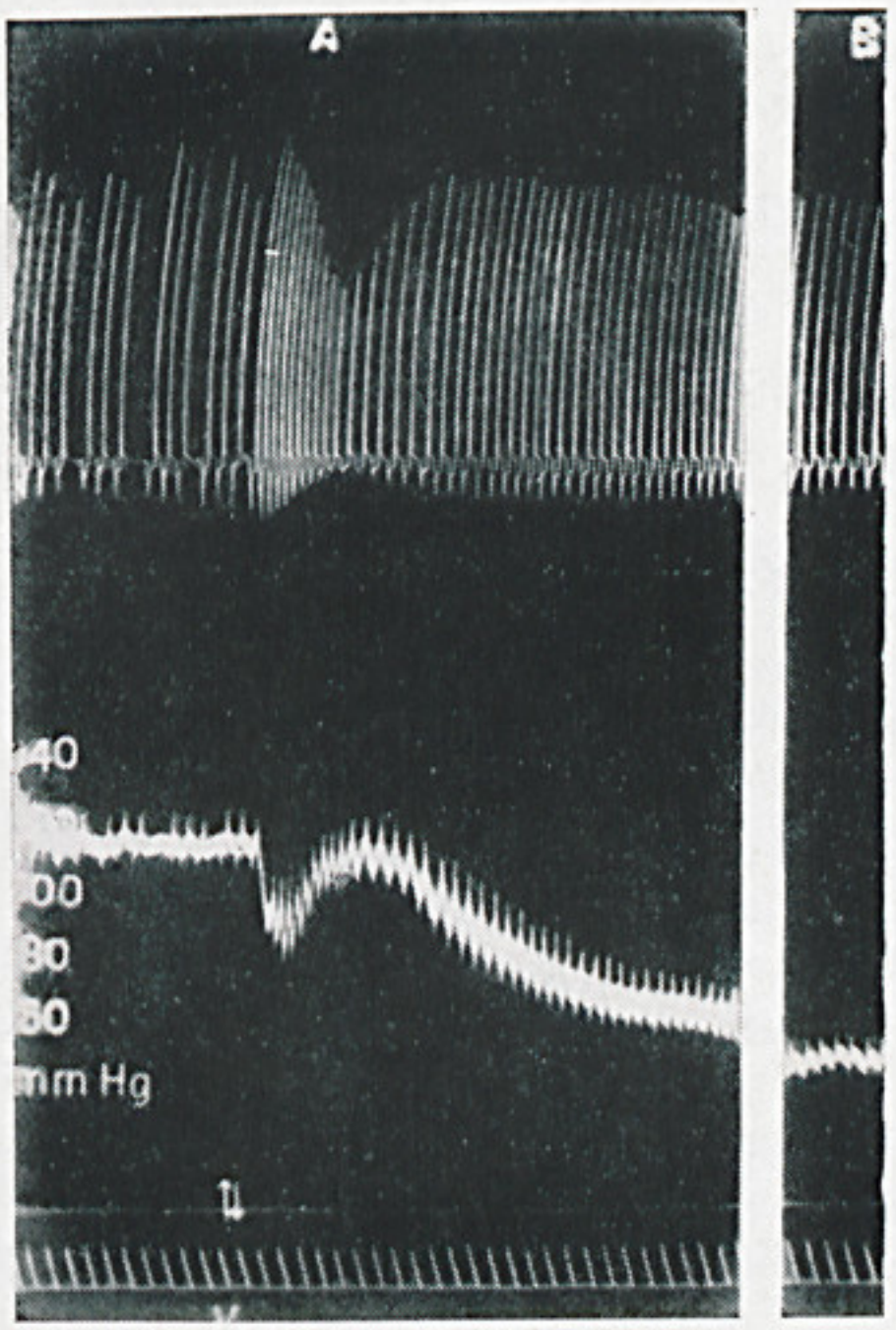

Fig. 4 — Dog, 6.0 Kg, sodium pentobarbital, both vagi sectioned. Records of respiration and blood pressure. At arrows, 0.25 mg/Kg of the venom of *C. d. terrificus* were injected. At B, record taken 7 minutes after A. Time 10/10 seconds.

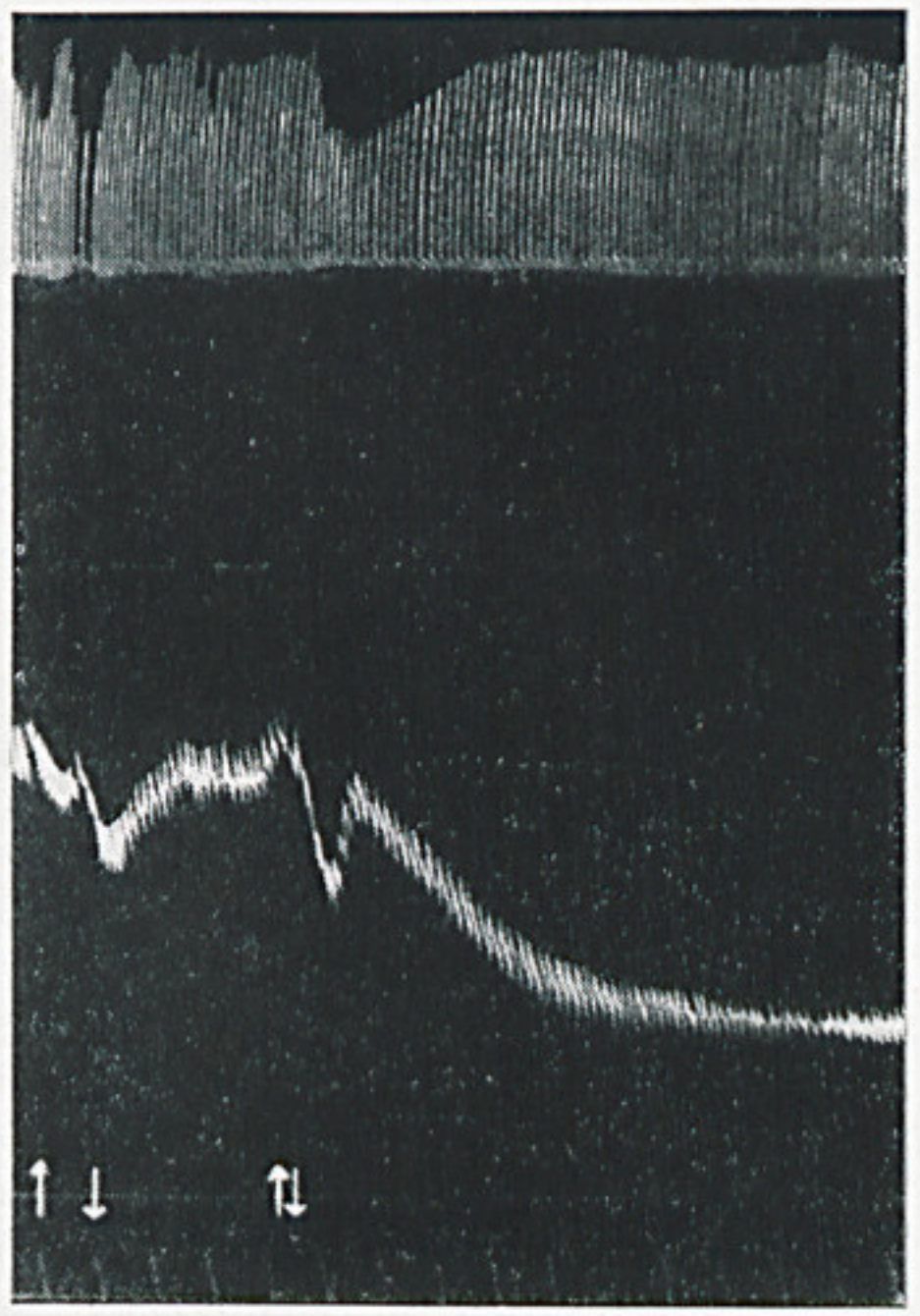

Fig. 5 — Dog, 6.5 Kg, sodium pentobarbital, both vagi and carotid sinus nerves sectioned. Records of respiration and blood pressure. First arrows, compression of the common carotid arteries was made, second arrows, 0.25 mg/Kg of the venom of *C. d. terrificus* were injected. Time 10/10 seconds.

much of the cardiovascular effects produced by the Indian cobra (*Naja naja*) venom to lysolecithin formation, an interpretation which can perhaps be questioned in view of the present results.

The triple effect, here reported, produced by the venom of *C. d. terrificus* on blood pressure, that is, the transient and abrupt fall of blood pressure which is followed by an hypertensive period of short duration and a secondary depression, was first described by Arthus in rabbits (1). He reported that he did not observe such a sequence of effects after administration of the other venoms he had studied. Vellard and Huidobro, on the other hand, showed that the venom of *C. d. terrificus* from some regions produces only depression of blood pressure, a finding that was confirmed by one of us (3) with the venom from rattlesnakes of Santiago del Estero, Argentina. The triple effect, as previously shown (3), is not supressed in dogs (i) injected with atropine, (ii) with their central nervous system destroyed by the procedure of Galvão and Pereira, (iii) with their autonomic ganglia blocked by hexamethonium and finally (iv) with their α adrenergic receptors blocked by dibenamine or chlorpromazine.

The brief period of apnoea can not be imputed to the respiratory stimulation caused by the venom as it was also observed in experiments in which only a small stimulation of the dog respiration was elicited. Moreover, in the experiments in which both vagi had been cut, the apnoea did not occur even in those

dogs whose respiration was intensely stimulated (two experiments in animals with the vagi and carotid sinus nerves cut). It seems, therefore, that this apnoea originates from the stimulation, in the lungs, of vagus nerve fibres which conduct inhibitory impulses to the respiratory centers. The brief period of apnoea evoked by nicotine also arises, according to Aviado (9), from stimulation of receptors in the lungs.

The respiratory stimulation caused by the venom was, in most cases, greatly reduced in those dogs whose vagi and carotid sinus nerves had been sectioned. This suggests that is must be, in part at least, reflexly generated, probably by an action of the venom on the chemoreceptors of the carotid and aortic bodies.

Summary

1. A comparative study of the acute effects produced by crotoxin and the venom of *C. d. terrificus* on blood pressure, haematocrit value and respiration was carried out in sodium pentobarbital anaesthetized dogs. The venom used in the investigation belonged to the variety devoid of crotamine.

2. Crotoxin was much less active than the venom in producing hypotension and in increasing the haematocrit value. The effects elicited by them on blood pressure were also qualitatively different from each other.

3. Respiration was not acutely modified by crotoxin. The venom, on the other hand, caused an intense and transient increase in the frequency and amplitude of the respiratory movements within a few seconds after its administration, a brief period of apnoea and one of tachypnoea which usually was very long lasting.

4. The results of the present investigation show, therefore, that the acute disturbances elicited by the venom on circulation and respiration can not be due to crotoxin or to crotamine. There are other venom components whose pharmacological actions must play an important role in *C. d. terrificus* envenomation, especially in the genesis of shock.

5. Some experiments were also made to clear up the mechanisms involved in the genesis of the respiratory disturbances that follow the administration of the venom. It was inferred from the results of these experiments that the brief period of apnoea originates from stimulation of receptors in the lungs while the respiratory stimulation is due to an activation of the chemoreceptors in the aortic and carotid sinus bodies, and to a direct action on the respiratory centers.

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