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63. PHARMACOLOGY OF THE VENOMS OF MEXICAN *CENTRUROIDES*

E. C. DEL POZO

Instituto de Salubridad y Enfermedades Tropicales, México

Recent reports from electrophysiological and biochemical work seem to show qualitative differences in scorpion venoms. Old descriptions of both clinical and experimental poisoning are similar and only quantitative discrepancies are found in observations corresponding to a wide variety of species from distant parts of the world. Differences in symptoms or signs of intoxication seemed to be due to variable amounts of venom or different content of active principles (1).

The general actions: muscular twitchings, ptyalism, progressive respiratory irregularities reaching respiratory paralysis in some cases, pilo-erection, mydriasis, blood pressure rise, signs of local pain, signs of laringeal constriction, are to be found in accounts of scorpion poisoning from any part of the world. However, the mechanism invoked to explain such actions varied very much.

At present, fundamental work is being done with scorpion venoms in many laboratories and agreement seems to be reached about the effects on nervous system and neuromuscular junctions. Nevertheless new discrepancies could be found in reports from electrophysiological studies. Different techniques are being used and one should keep in mind that discrepancies are early and stimulating aspects of original research.

In spite of the fact that new paths of research in pharmacology of scorpion venoms may lead to the discovery of a common mechanism of action, it is essential to mention always the scorpion species used in each work.

The description that follows is limited to pharmacological properties of venom from Mexican *C e n t r u r o i d e s*, and it is based on works from my laboratory. Our comparative studies of the actions of venoms of *C. suffusus suffusus* Pocock, *C. noxius* Hoffmann, *C. limpidus tecomanus* Hoffmann and *C. limpidus limpidus* Karsch, grant a common consideration. Only quantitative differences have been found. Larger doses of the less active venoms reproduce the effects of smaller doses of the more active ones.

MUSCULAR EFFECTS — One of the most immediate effects of intravenous injections of scorpion venom in cats, dogs, mice and rats is the appearance of generalized muscular twitchings and fascicular contractions. This activity originates in the spinal cord. It persists after section of the brain stem, deafferentation or transection of the spinal cord. The fascicular contractions below the level of the section are more marked than above, but they disappear completely when the spinal cord is destroyed or the motor nerves are cut (Fig. 1).

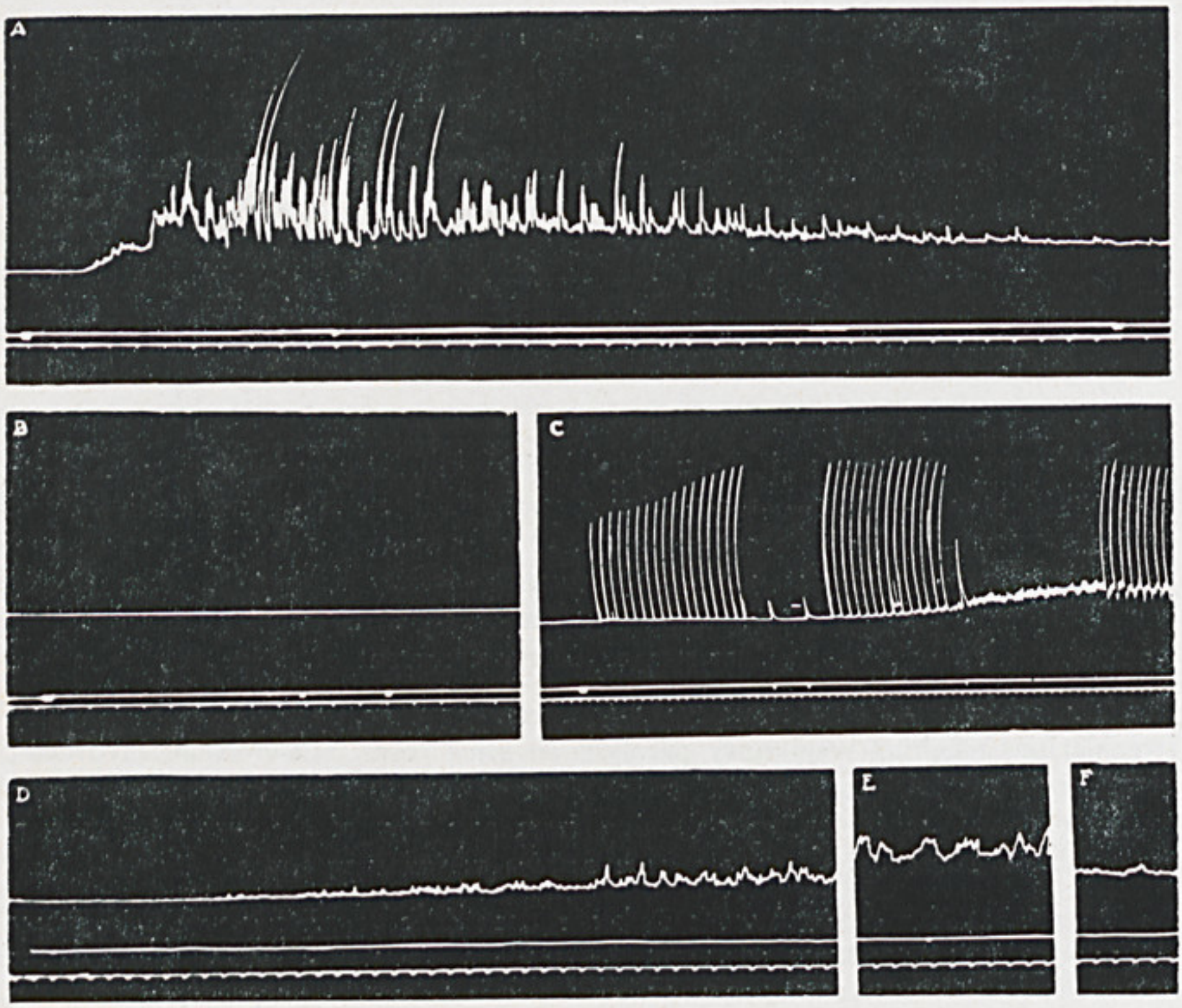

Fig. 1 — A: Muscular effects of an intravenous injection of scorpion venom. B: No effect is obtained when an equal amount of venom is injected after the muscular nerve was cut. (This and Figures 3 to 7 taken from del Pozo and Anguiano (2)).

Muscular twitchings and fibrillations are also obtained if the venom is applied directly to one muscle or injected intrarterially even with the corresponding nerve cut. This muscular activity does not occur when time has been allowed for Wallerian degeneration after denervation. Local application of venom to a muscular nerve trunk does not produce any effects on the muscles (2).

The local action of the venom takes place on the muscular end-plate region. This inference was confirmed when electrical records were taken from muscle and nerve. Repetitive potentials were found following single shocks applied to the nerve centrally cut. This repetitive activity is conducted antidromically in the nerve and disappears when this nerve is disconnected from the muscle. When records were taken from anterior and dorsal roots, the repetitive activity was seen in the former and not in the latter (4) (Fig. 2).

Big and long-lasting contractions are obtained when single shocks are applied to muscles under the action of scorpion venom. When successive responses are provoked at short intervals, the contractions are progressively smaller in amplitude and duration. Electrical recordings show at the same time a gradual diminution of the repetitive activity (Figs. 3 and 4).

According to this evidence the great and long muscular responses to single shocks correspond to short tetanic contractions consecutive to the repetitive activity provoked at the end-plate regions of poisoned muscles by the arrival of single impulses.

These effects on time and duration of muscular contraction and the influence of repetitive stimulation have been extensively analyzed at different frequencies of stimulation. The graphs of tension development of the tetanic contractions shows mechanograms resembling those obtained when higher frequencies of stimulation are applied to normal muscles (2) (Fig. 5).

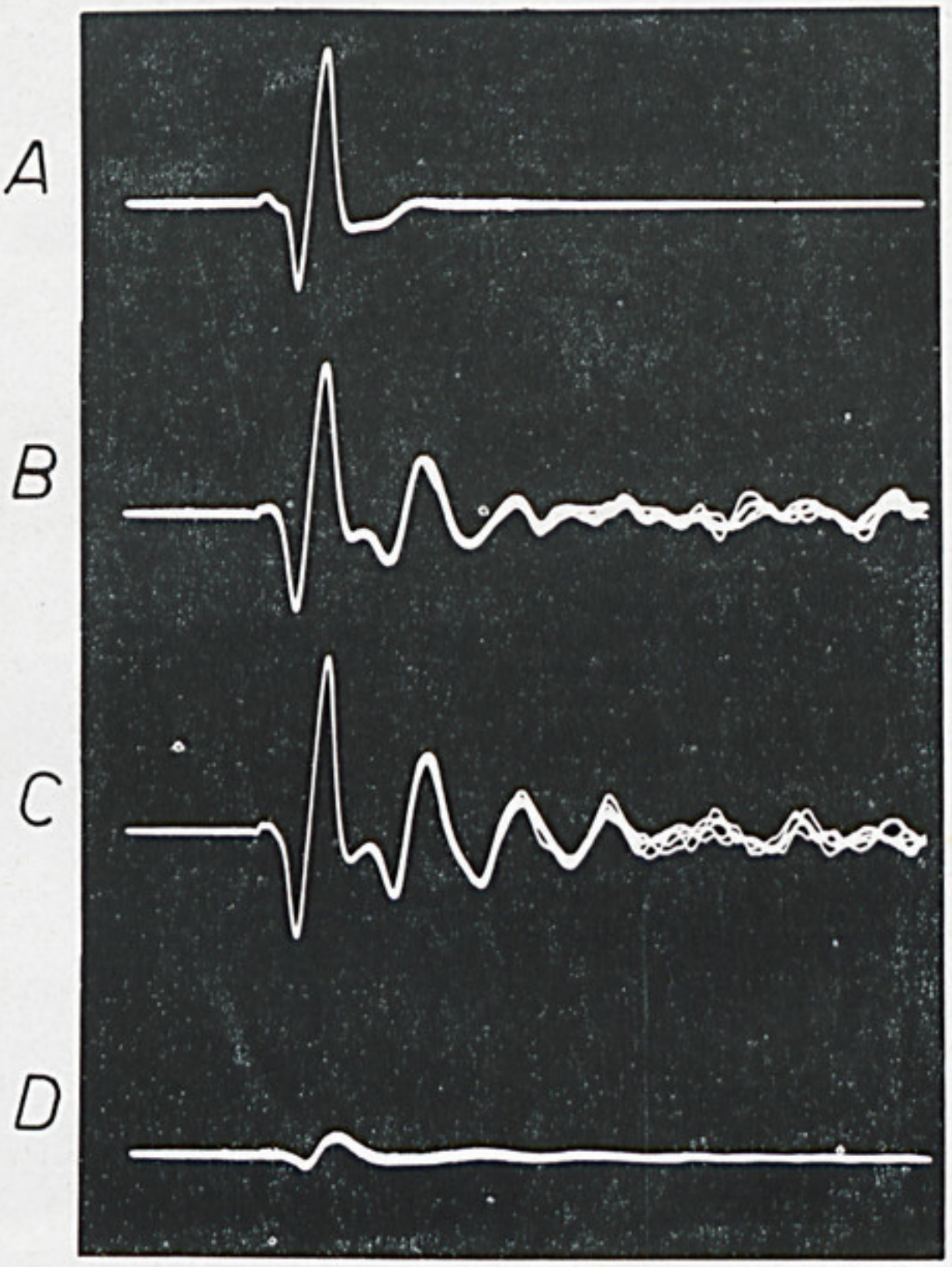

Fig. 2 — Electrical recording from gastrocnemius muscle of the cat. Five superimposed responses to shocks applied to the nerve in each segment. A, before; B to D, after successive dosis of scorpion venom. (This and fig. 8 taken from del Pozo, Salas and Pacheco, in press).

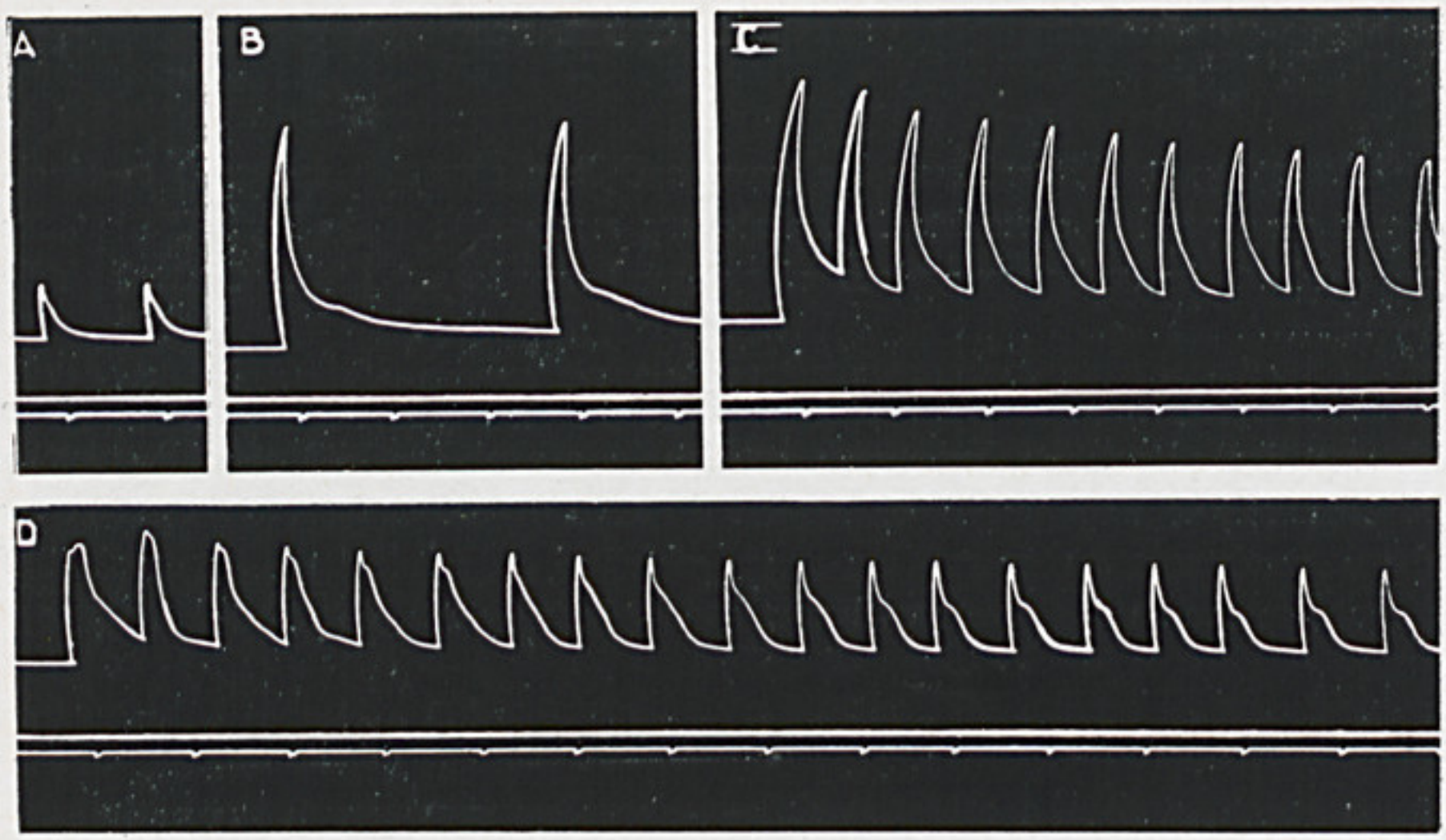

Fig. 3 — Effect of scorpion venom on the amplitude and duration of muscular responses. Maximal contractions of gastrocnemius of the cat, before (A) and after the injection of the venom (B). In successive contractions, the amplitude and duration are progressively reduced (C). (D), 20 minutes later.

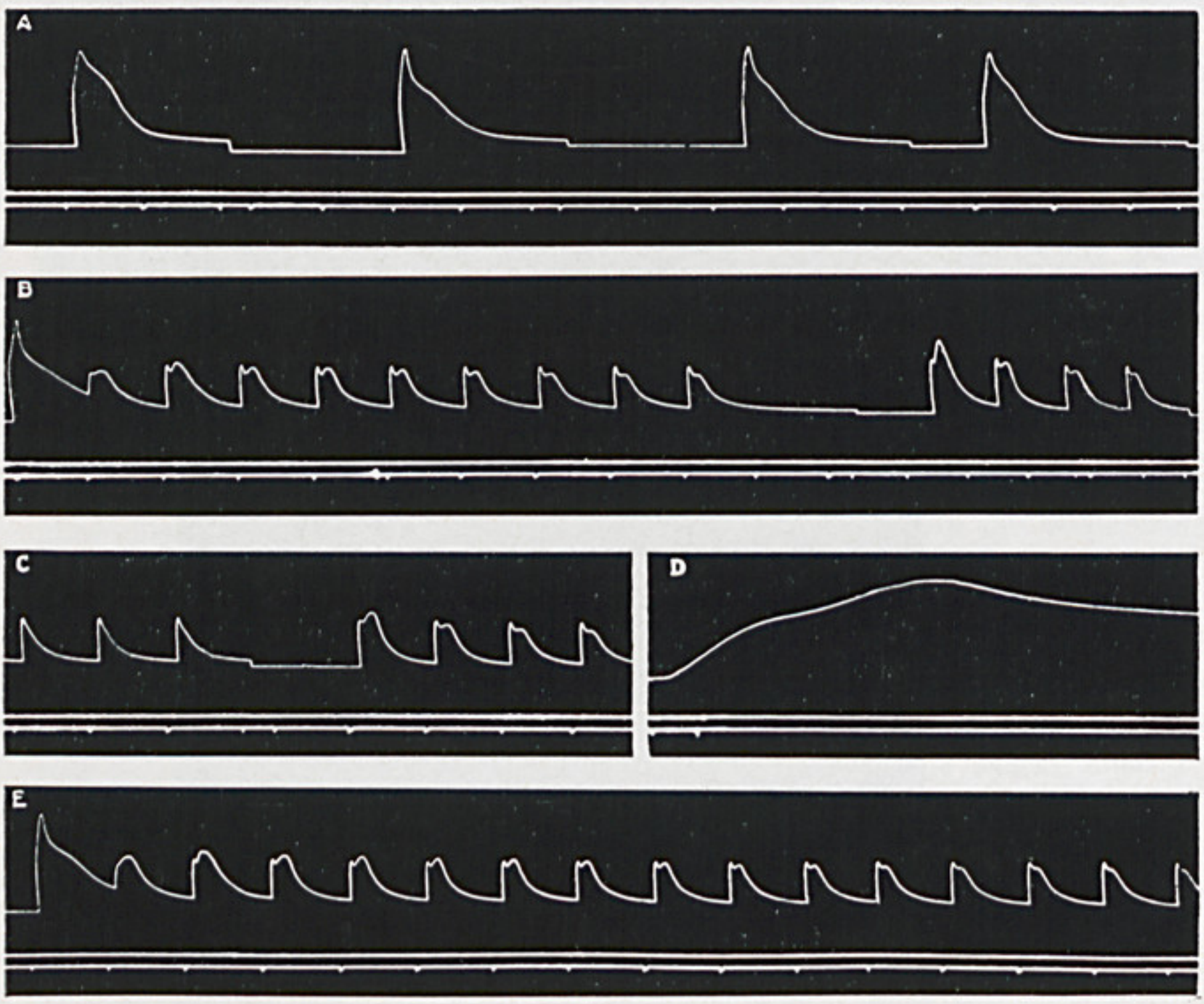

Fig. 4 — The length of the interval between stimulations change the type of responses in a muscle under the action of scorpion venom. A, 3 minutes interval between shocks. B, one second intervals.

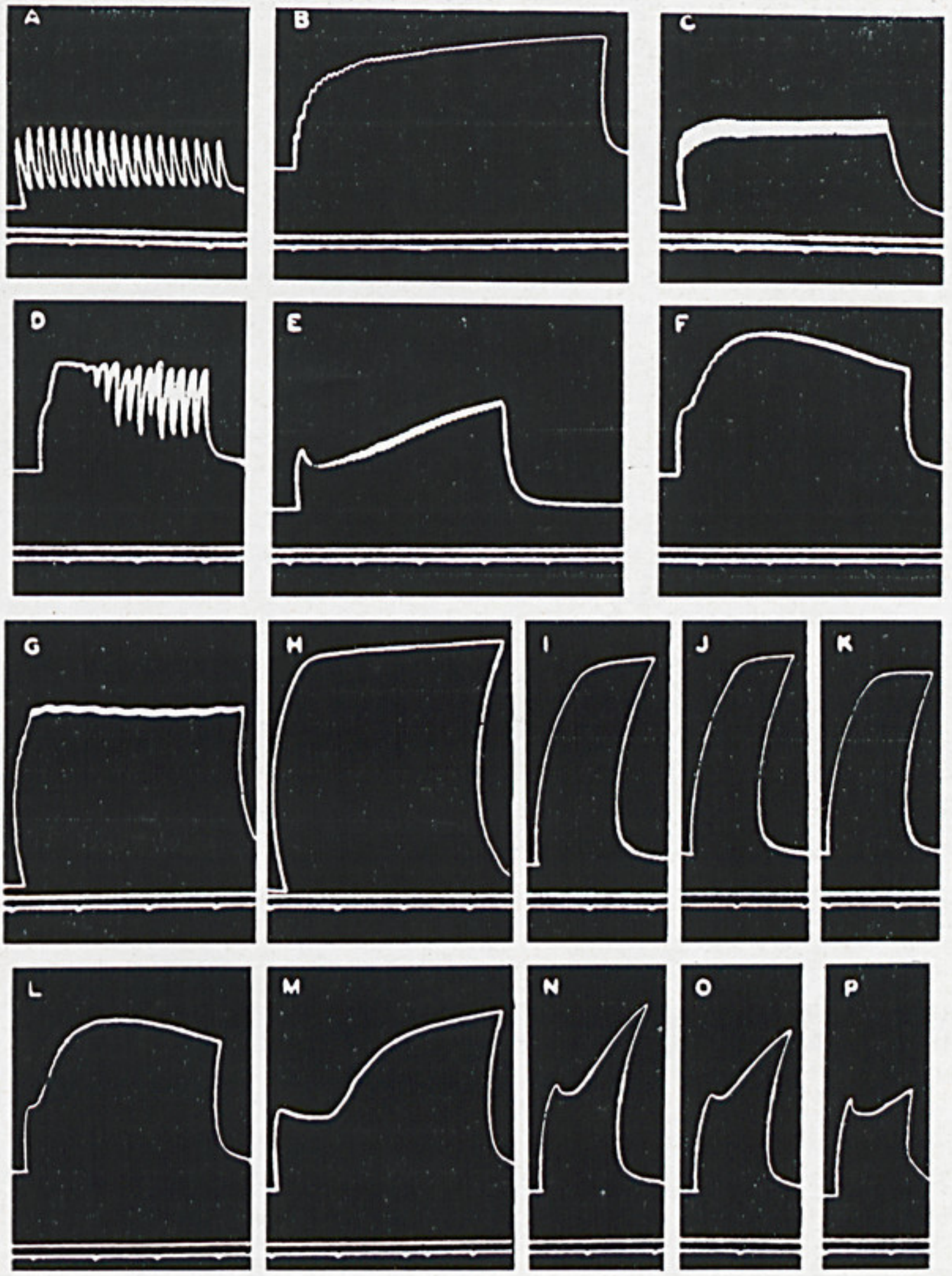

Fig. 5 — Effect of scorpion venom on muscular responses to high frequency stimulation. Pairs of responses before and after injection of the venom to stimulation to the following frequencies per second: A and D, 5; B and E, 13; C and F, 17; G and L, 25; H and M, 30; I and N, 60; J and O, 120; and K and P, 200. Gastrocnemius of the cat.

The tetanizing minimal frequency of the stimulus for one particular muscle is smaller when poisoned than for a normal one, but after the initial complete tetanus the individual responses to each shock appear on the graph. This peculiar development of the contraction is the opposite of the normal graph obtained at the minimal tetanizing frequency, i.e., initial individual response that progressively

fuse in a complete tetanus. The long lasting initial contractions of the poisoned muscle and the progressive shortening of the successive responses account for this phenomenon (Fig. 6).

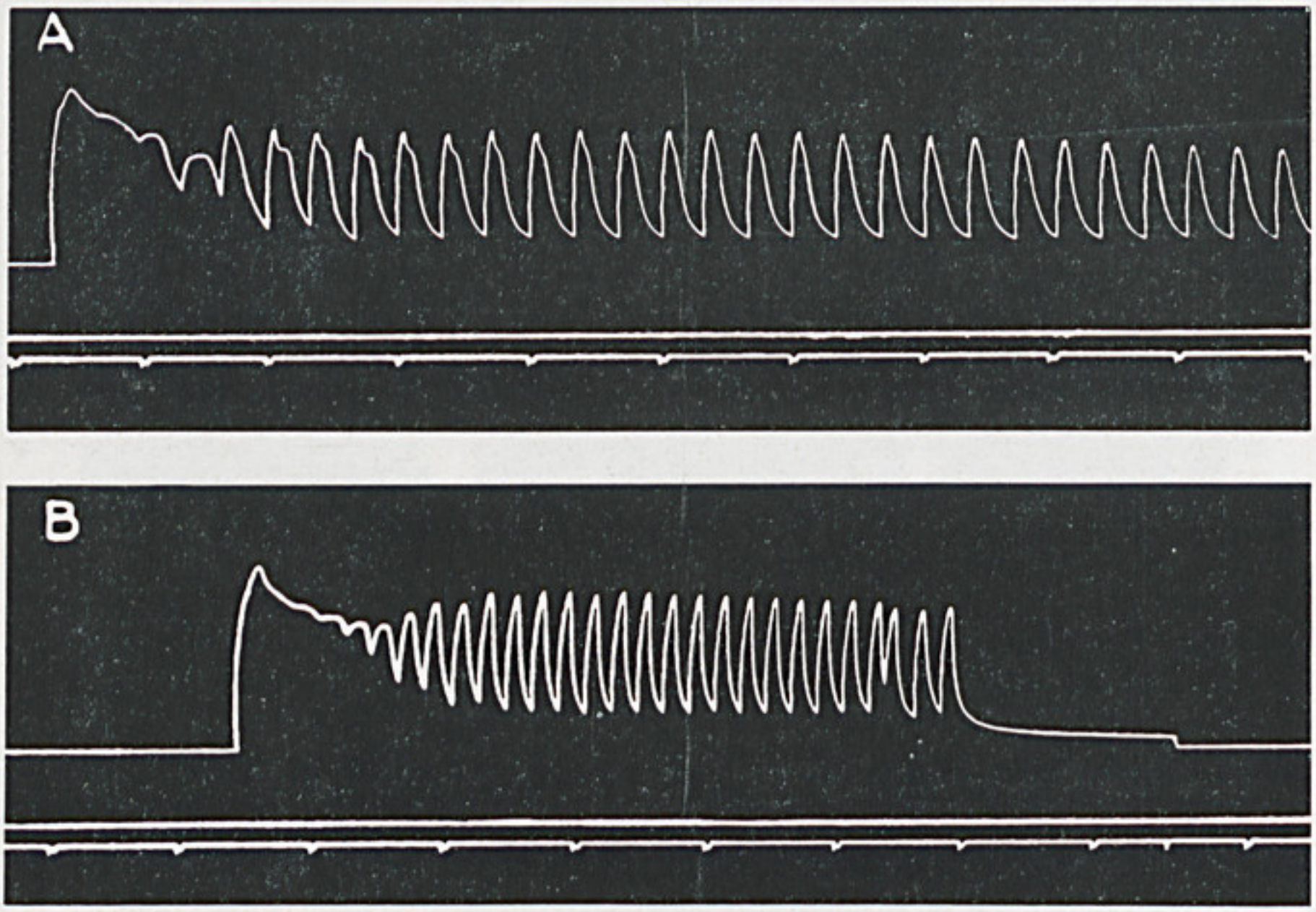

Fig. 6 — Inversion of the stages of the responses to stimulation at a minimal tetanizing frequency in a muscle under scorpion venom. A, 3 per second; B, 5 per second.

When neuromuscular transmission has been blocked by curare, scorpion venom given by intravenous injection decurarizes (2). However, large doses of the venom produce a complete block of neuromuscular transmission. This block is not suppressed by either prostigmin or curare. The muscular responses to direct electrical or acetylcholine stimulation are preserved (Figs. 7 and 8).

Scorpion venom displays anti-cholinesterase activity as shown by our experiments testing the fall of blood pressure produced by fixed amounts of acetylcholine after hydrolysis with blood serum with and without venom.

This property was also assayed comparatively with eserine on frog abdominal muscles (5). The anticholinesterase activity of different venoms was found to be slight and without correlation with toxicity or the muscle activating properties.

A study of the effects of venom (*C. noxius* Hoffmann) on cholinesterases was examined on the isolated intestines of guinea-pigs and rabbits. It was found that the venom only inhibit cholinesterases both from human serum (pseudo) and from the caudate nucleus of rabbits (specific) when present in very high concentrations (6).

In brief, the muscular effects of scorpion poisoning are produced by two actions of the venom: one, central, located on the spinal cord, and the other, peripheral, on the neuromuscular junctions. Both actions take place at regions

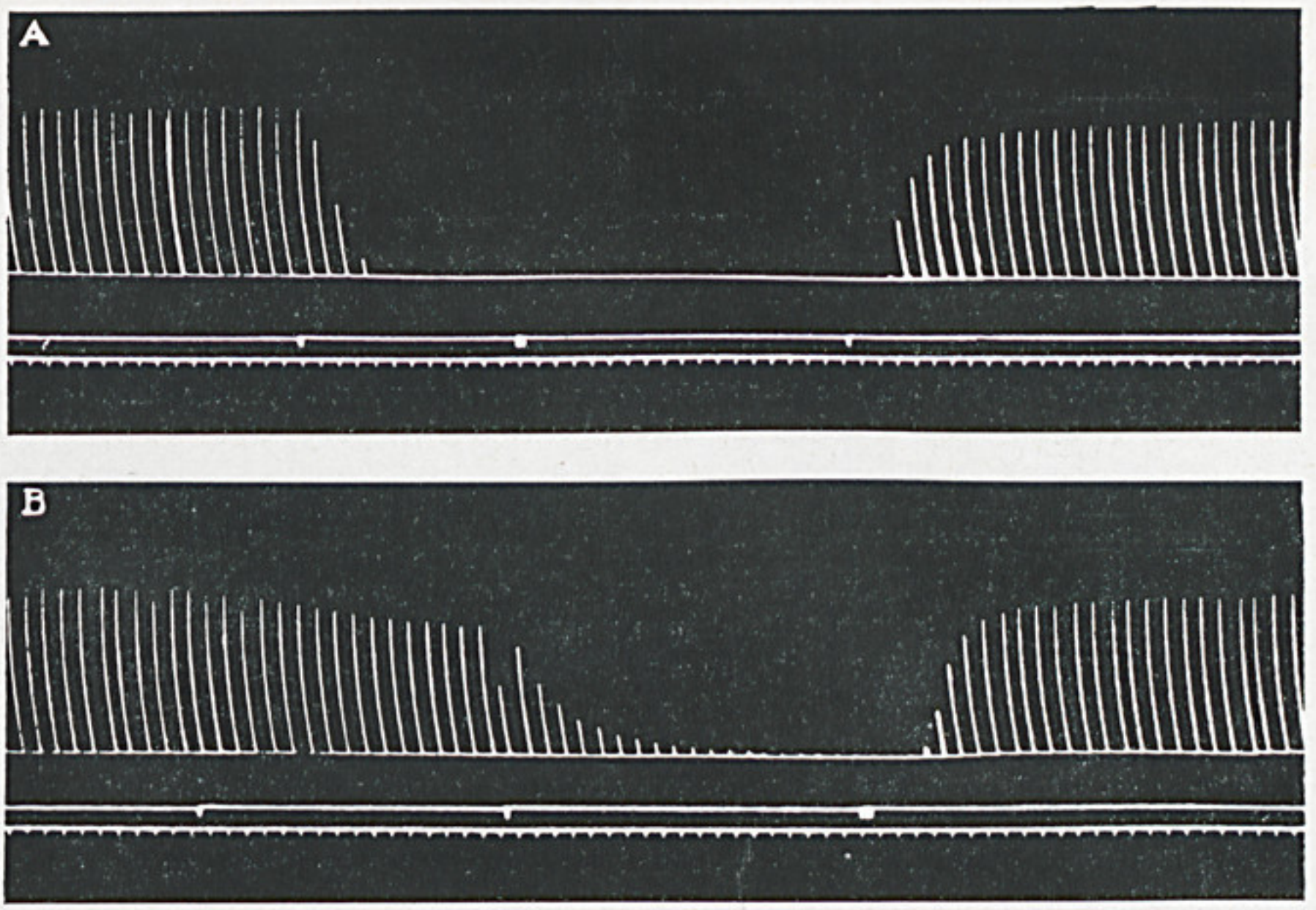

Fig. 7 — Decurarization by scorpion venom (B) and by prostigmine (A). The first signal in both segments correspond to the injection of curare. The second in B to a second injection of curare (in A to atropine). The third signal in B marks the time of the intravenous injection of scorpion venom. (In A, prostigmine).

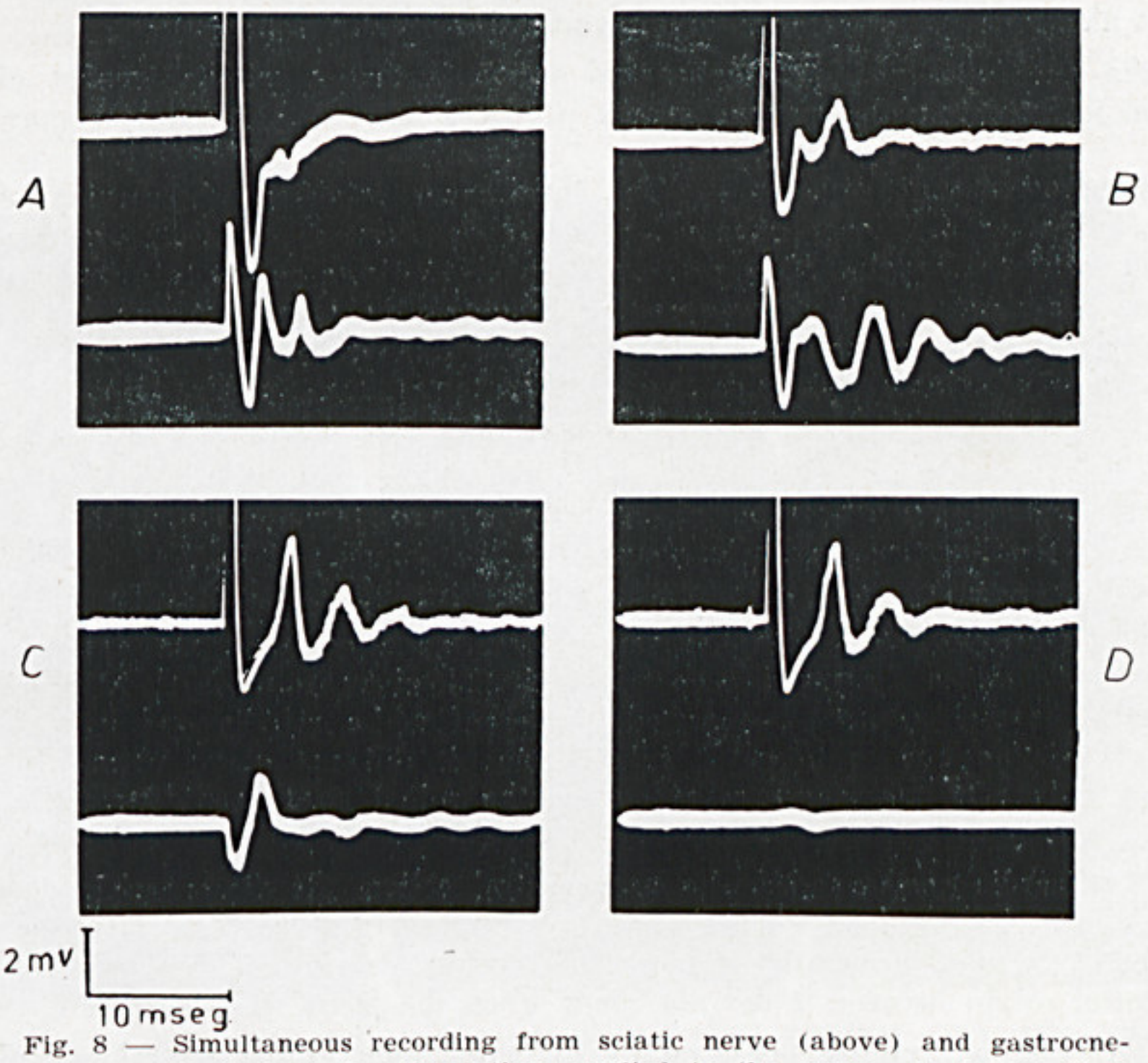

Fig. 8 — Simultaneous recording from sciatic nerve (above) and gastrocnemius muscle (below) to stimulations applied to the nerve. A, before, B, 1 minute after injection of scorpion venom. C, 5 minutes later. D, after curare.

of excitable membranes, and it is reasonable to assume that the venom affects the permeability of these membranes and change the ionic equilibrium between both sides of the same.

Macroelectrode recordings from the end-plate regions of the sartorius muscle of a cat showed a progressive inversion of the local potential under the action of scorpion venom (7). This is a direct evidence of changes in ionic distribution produced by the scorpion toxins.

Respiratory effects — The accidental or experimental poisoning with scorpion venom produce marked irregularity in different animals both in frequency and amplitude of the respiratory movements which in cases of strong intoxication reaches a Cheyne-Stokes type rhythm and finally paralysis. The central origin of this paralysis was attested by keeping the animals (cats) alive after the paralysis by means of artificial respiration brought about by rhythmic stimulation of both phrenic nerves. In these experiments, to our knowledge, was for the first time applied what afterwards was introduced under the name of *electrophrenic respiration.* The paper published in 1945 refuted the hypothesis of curarization as the origin of the respiratory paralysis (8).

Bronchiolar obstructions by abundant secretions, laryngeal and bronchiolar muscles contractions contribute to the respiratory distress but are not the cause of the asphixia. Same may be said of the fascicular contractions that appear in respiratory as well as in all skeletal muscles. Periodic rhythms of respiration from central origin can be perceived among the background of irregular jerks up to the time of appearance of Cheyne-Stokes type rhythms.

An additional proof of the central action is that a minute amount of the venom injected into the *cisterna magna* produces immediate respiratory paralysis.

Cardiovascular effects — The intravenous injection of scorpion venom produce in cats and other mammalians an initial increase of blood pressure and simultaneous bradicardia. The pressure then comes down slowly to normal values. During the periods of cyclic respiration, increases in blood pressure accompany the periods of apnea and blood pressure falls during hyperventilation. Finally, if the dose of venom was high the pressure comes down gradually to zero (9).

The initial increase in blood pressure long time ascribed to a peripheral action of the venom is due to effets on the spinal vaso-constrictor presynaptic neurons and to the liberation of epinephrine also through stimulation of the adrenal activating pre-ganglionic neurons of the spinal cord.

Spinal animals after total destruction of the brain, give equal or higher blood pressure increases with the venom, but the effect does not appear when the spinal cord is destroyed even when the blood pressure level is previously raised to normal values. Denervated pinnae of white rabbits and cats showed vaso-constriction due to liberated epinephrine; it did not occur when the sympathetic fibers to the glands were cut or the spinal cord was destroyed (Figs. 9 and 10).

Bradicardia corresponded also to a central action. It disappeared when the medula was destroyed or when the vagi were cut. In these cases the heart was slightly accelerated under the influence of scorpion venom. This effect was also of central origin because it did not occur when the heart was denervated.

The vascular reflexes produced by the stimulation of the central end of the sciatic nerves peripherally cut were not modified by scorpion venom on curarized

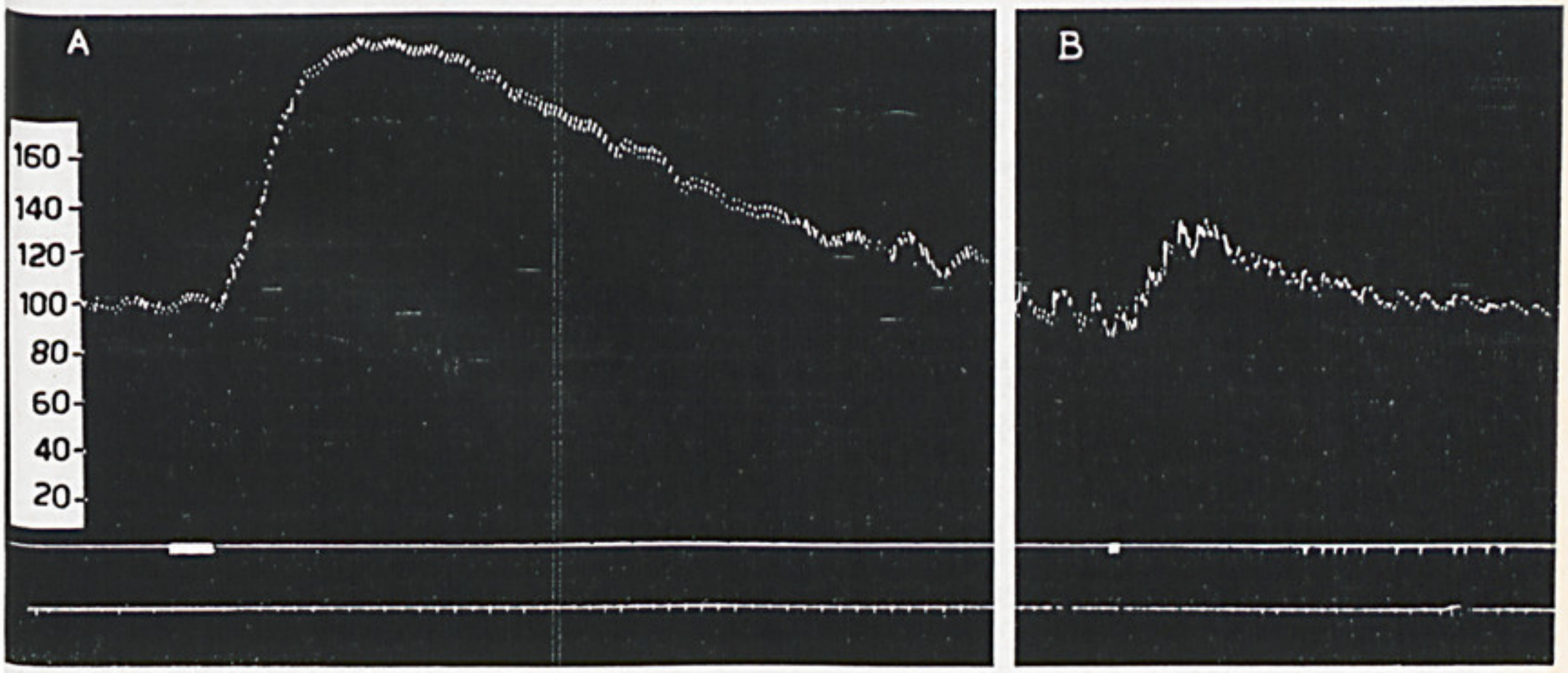

Fig. 9 — Increases of blood pressure produced by a first (A) and a second (B) intravenous injection of scorpion venom in a spinal cat by destruction of the brain. (This and the following figure are taken from del Pozo, Anguiano and González (9)).

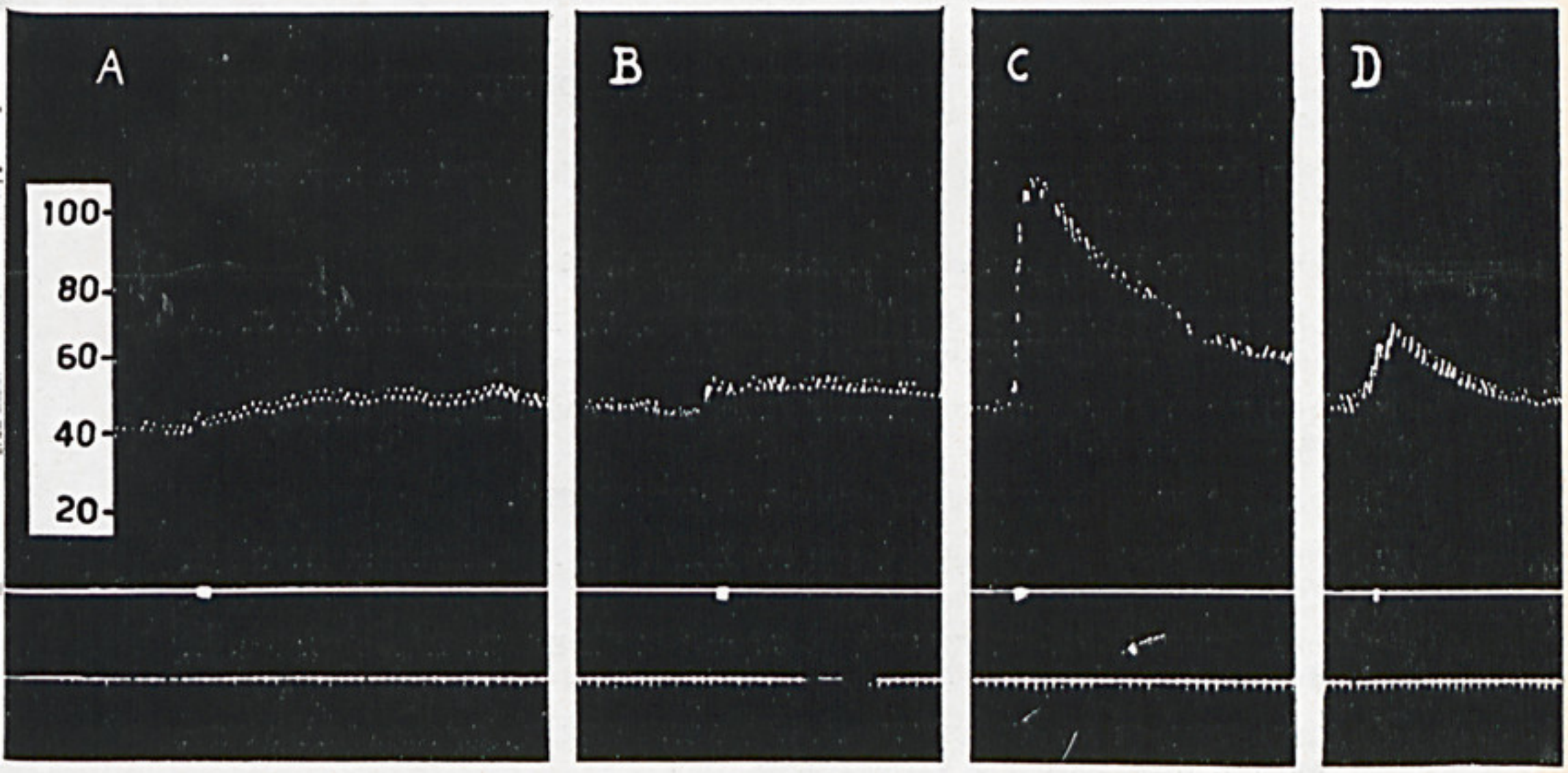

Fig. 10 — Scorpion venom does not produce increase of blood pressure when brain and spinal chord are destroyed. A, first injection of venom; B, injection of an equal volume of saline; C, injection of 10 mcg of epinephrine; D, 5 mcg of epinephrine.

animals. This dissociation between the tonic vasopressor direct effects and the reflex responses is an old physiological finding reported by Sherrington in 1906 (10), Porter in 1910 (11) and Langley in 1924 (12).

OTHER EFFECTS — Other effects of the scorpion venom such as ptyalism, mydriasis, piloerection are also to be explained by central action on the sympathetic pre-ganglionic neurons because those effects are greatly reduced or disappear completely by denervation.

We have seen that the rich pharmacological actions of scorpion venoms seem to derive from a fundamental common mechanism present at different anatomical regions. We have discovered already that this venom acts on the typical places so-called *centers* and at neuromuscular junctions. We know that it works by increasing the permeability of excitable membranes as shown by the inversion of end-plate potential (7). Others investigators have reached similar results with finer techniques (13).

In addition, valuable biochemical work is being done in several laboratories for the isolation of scorpion toxins; we may be hopeful that now we are on the road to a basic understanding of the mechanism of action of these peculiar venoms.

This first International Symposium has brought together the people working on the same problems in all parts of the earth. It will be an historical event. The importance of this meeting is increased because we meet at this famous Butantan Institute, the leader of the venomological studies. We pay tribute to the illustrious Vital Brazil, inspired founder of the Institute and to all the group of workers that have followed his steps in this fascinating path of research.

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Discussion

H. Edery: "All these features you described on the neuromuscular transmission such as twitchings, and fasciculations are also produced by anticholinesterase organophosphorous compounds. I learnt from your paper that you tested the venom for anticholinesterase activity and it has a high concentration. I would like to ask if you would accept the idea that at least part of the effect on neuromuscular transmission were due to inhibition of cholinesterase at the end-plate level. When you inject a substance intraarterially, you do get high level concentrations. And thinking in this way of inhibition of cholinesterase, I would be keen to know the effects of the so-called reactivations of cholinesterase on the block produced by the venom."

E. del Pozo: "We did find anticholinesterase activity and we thought that this could be the explanation for the muscular effects of scorpion venom. However, when tested that possibility we found that the anticholinesterase effect was slight and did not keep relation or correlation with the muscle activating properties. For this last testing we compared venoms from different scorpion species with regards to anticholinesterase activity, toxicity and muscle activating properties. One particular venom could have less anticholinesterase and more muscle activating properties than other."

P. Efrati: "Also my experience concerns mainly stings by *Leiurus quinquestriatus*, I think, at least two clinical signs, observed in general envenomation, could support the observation of E. C. del Pozo about the influence of scorpion venom on the spinal cord: urinary retention and priapism, observed very often indeed."