# Effects of *Latrodectus mactans tredecimguttatus* venom on the dynamics of vertebrate voluntary muscles

MICHELE MAROLI and SERGIO BETTINI

*Department of Parasitology*

**Summary.** — The effects of Black Widow Spider (*Latrodectus mactans tredecimguttatus*) crude gland venom have been studied on frog and mouse neuromuscular preparations.

Fibrillation and contracture, varying in intensity according to venom concentration, have been observed. These phenomena are enhanced by application of eserine. The addition of ACh to the venom potentiates its effect. Frog isolated voluntary muscles treated with venom release a contracturing substance in the bath, probably ACh. Denervation and curare neutralize the venom effect. Application of antivenin, as opposed to washing, induced complete recovery of the preparations. Gland lumen venom causes effects similar to those induced by comparable concentrations of crude gland extract. The venom of the Black Widow Spider induces no alteration in the neuromuscular cockroach preparation.

The probable mode of action of the venom, *i. e.* massive release of ACh at the level of neuromuscular synapses, is discussed on the base of recent results obtained by various authors. At the light of such a mode of action a tentative explanation of the muscular symptomatology in vertebrate envenomation is given.

**Riassunto** (*Effetti del veleno di* Latrodectus mactans tredecimguttatus *sulla dinamica dei muscoli volontari di vertebrato*). — È stato studiato l'effetto del veleno di *Latrodectus mactans tredecimguttatus* su preparati neuromuscolari isolati di rana (m. tibiale àntico) e di topo (retto addominale); sono stati osservati treni di fibrillazioni e contrattura, (registrati con trasduttore RCA 5734), fenomeni che variano di intensità e durata con il variare delle concentrazioni di veleno.

Tali fenomeni sono accentuati da applicazione di eserina. L'aggiunta di ACh al veleno ne potenzia l'effetto. Muscoli volontari isolati di rana, trattati con veleno, liberano nel bagno una sostanza contratturante, probabilmente ACh. La denervazione ed il curaro neutralizzano l'effetto del veleno. L'aggiunta di siero immune ripristina l'attività normale del preparato, effetto che non si ottiene con il lavaggio. Il veleno estratto con capillare di vetro dal lume ghiandolare causa effetti simili a quelli provocati da una dose di veleno pari a quello ottenuto omogenizzando un eguale numero di ghiandole. Il veleno di *Latrodectus* non provoca alcuna alterazione sull'attività del preparato neuro-muscolare di blatta (muscoli estensori del trocantere). Il probabile meccanismo d'azione del veleno, cioè la massiva liberazione di ACh a livello delle placche neuro-muscolari, viene discusso in base ai recenti risultati ottenuti da vari Autori. Alla luce di tale meccanismo d'azione viene presentato un tentativo di spiegazione della sintomatologia muscolare nell'avvelenemento del vertebrato.

---

The symptomatology of Black Widow Spider (BWS) bite (latrodectism) is mainly neurotoxic. A review of the literature concerning latrodectism symptomatology has been published by BETTINI & CANTORE (1959). It appears that the most common symptoms referring to muscular impairement are opistotonus, spasm of dorsal and neck muscles, spastic contractions of the large muscles of the limbs, tremors, and contracture of abdominal muscles simulating acute abdomen.

These symptoms led several authors to investigate upon the effects of BWS venom on neuromuscular preparations. TROISE (1929) referred that toad *m. sartorius* shows evident fibrillation if bathed in a diluted crude gland extract (CGE). Working on a rat neuromuscular preparation (not better identified by the authors), D'AMOUR, BECKER & VAN RIPER (1936) reported that CGE does not show any effect on the nerve trunk, nor on the muscle or neuromuscular junction. SAMPAYO (1944) also obtained negative results on a toad *sartorius* neuromuscular preparation and on the denervated muscle. TROISE (1928) and SAMPAYO (1942) using anaestetized and spinal animals concluded that CGE acts on the spinal cord (exaggeration of muscular symptoms in spinal animals), and not on motor nerves and on muscles. However, further research by CANTORE (1958) on a rat nerve-diaphragm preparation and by RUSSELL & LONG (1961) and RUSSELL, O'BRIEN & INABA (1961) on an analogous preparation from guinea pig, showed that CGE causes a definite neuromuscular block. These findings were recently substantiated and the mode of action of the venom was clarified by LONGENECKER *et al.* (1970) and by CLARK *et al.* (1970). These authors demonstrated by intracellular recordings of frog *sartorius* fibres, that CGE induces very high

rates of minipotentials release even at zero calcium concentration, and showed by electron microscopy that, following the minipotential release, nerve endings appear completely depleted of ACh vescicles.

The aim of the present work was to study the effects of BWS venom on voluntary muscles in order to throw light on part of the symptomatology of latrodectism in vertebrates.

## *Materials and Methods*

The BWS venom used in our experiments was obtained either by extraction from gland homogenates (CGE) following the technique of d'AJELLO, MAURO & BETTINI (1969) or directly by piercing the gland with a microcapillary and withdrawing the venom from the gland lumen (GLV) according to the technique of MAJORI, BETTINI & CASAGLIA (in press).

The concentrated CGE was prepared by homogenizing 50 pairs of glands in 1 ml of buffered saline, the protein content (LOWRY, *et al.* 1951) of the homogenate was about 9.5 mg/ml. The extract was distributed in 10 small ampouls and kept at — 20°C. The vertebrate neuromuscular preparations employed were the frog *n. peronierus* connected with *m. tibialis anticus longus* and the mouse isolated *m. rectus abdominis*. A Ringer physiological solution was employed for the frog (NaCl 6.2 g/l, KCl 0.13 g/l, $PO_4H_2Na$ 0.019 g/l, $CaCl_2$ 0.11 g/l, $CO_3HNa$ 0.3 g/l, glucose 1 g/l) and a Tyrode solution for the mouse (NaCl 8 g/l, KCl 0.2 g/l, $CaCl_2$ 0.2 g/l, $MgCl_2$ 0.1 g/l, $NaH_2PO_4$ 0.1 g/l, $CO_3HNa$ 0.5 g/l, glucose 1 g/l).

For comparative experiments on insects we employed 20-25 day old male *Periplaneta americana* bred in the laboratory under standard conditions. The cockroach neuromuscular preparation employed was the nerve 4 of the mesothoracic ganglion, connected with the extensor muscle of the trochanter. The physiological solution used has been that of Roeder (NaCl 9.0 g/l, KCl 0.2 g/l, $CaCl_2$ 0.2 g/l, glucose 4 g/l, 10 ml/l of phosphate buffer at pH 7.2).

The physiological solutions were kept oxygenated ($O_2 + 5\ \%\ CO_2$). Platinum electrodes were utilized. A Mugel S-50 stimulator giving square pulses of variable duration, amplitude and frequency, a transducer (RCA 5734) and a Galileo double track ink-pen recorder Mod. RI2a were used (*). In one track of the recorder the trace corresponded to 2 cm/g, in the other to 22 cm/g; speed 1 mm/sec. Fine movements of the preparation and of the electrodes were accomplished by the use of two Narishige micromanipulators. The antivenin was prepared by injecting scalar doses of extract of

(*) We are grateful to Mr. G. Massetti for his highly qualified technical assistance for the electronic apparatus.

BWS cephalothorax homogenate into sheep. A dose of 0.5 ml of immune serum applied subcutaneously protects a 250 g guinea pig injected with 5 $DL_{50}$ (60 μg of protein) of CGE. A known weight of lyophilized antiserum corresponding to a volume of 150 μl of whole serum was dissolved in 45 μl of water.

*Results*

The application of CGE at a dilution of 50 μl of the concentrated extract/ml of saline determines a characteristic activity, on the frog *m. tibialis a. l.*, as shown in Fig. 1A. The onset of fibrillation occurs about 2-3 min after the application. These fibrillations are at first small in amplitude, but after 10 min they become 4-5 fold larger. Typical bursts of fibrillations take place throughout the period of muscular activity induced by CGE. After 30 min the activity decreases rapidly and disappears at 45 min. Many trials with the same type of preparation constantly gave the same pattern, with small differences in the frequency of fibrillations and duration of the activity. However, when a lower concentration of CGE (1.6 μl/ml) was applied, the fibrillation followed a similar trend but had longer duration, and lower frequency and intensity. Other trials performed by applying GLV, obtained from 2 pairs of glands and diluted so as to equal the dose of 2 μl of concentrated CGE/ml, to frog *m. tibialis a. l.* caused a sequence of events in all similar to those observed following application of CGE 2 μl/ml.

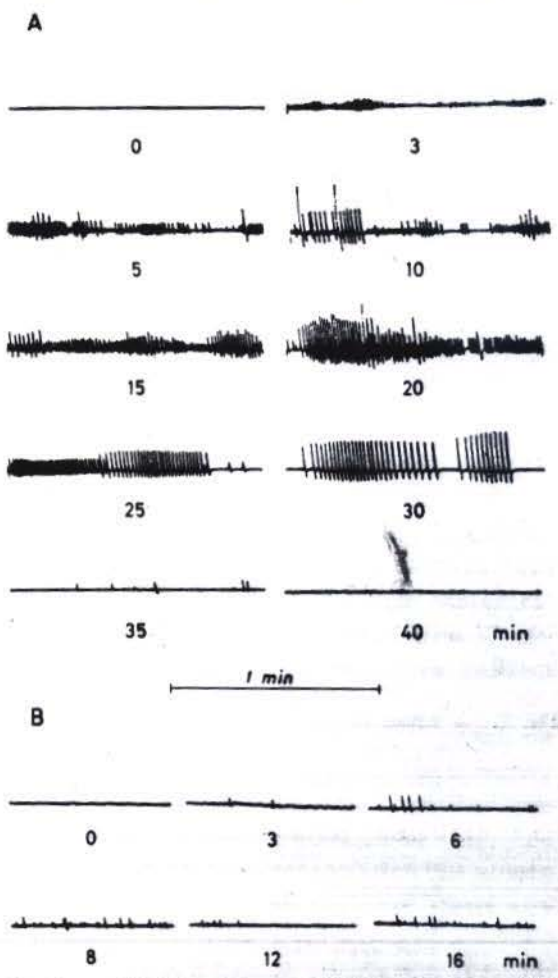



Fig. 1. — Motility of frog *m. tibialis anticus longus* (A) and mouse *m. rectus abdominis* (B) following application of CGE (50 μl of conc. extract/ml).

Application of CGE at a concentration of 50 µl/ml on mouse *m. rectus abdominis* induces similar muscular activity, although less intense and of shorter duration (about 15 min), as shown in Fig. 1B.

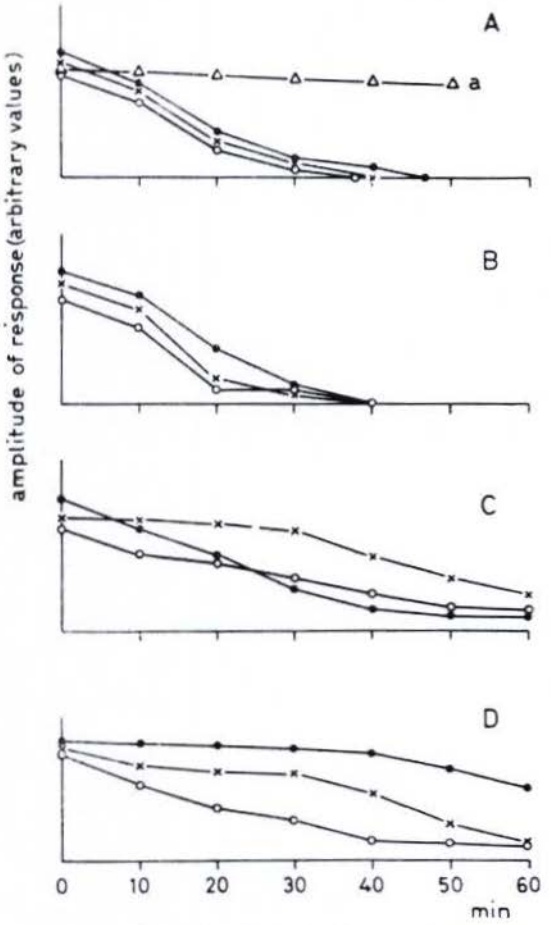



Fig. 2. — Effect of CGE on indirectly induced contractions of frog *m. tibialis anticus longus:* (A) after application of 25 µl of conc. extract/ml, stimulus frequency 6/min (*a:* control); (B) after application of same concentration of CGE as in (A), frequency 3/10 min; (C) after application of 0.1 µl of conc. extract/ml, stimulus frequency 6/min; (D) after application of same concentration of CGE as in (C), stimulus frequency 3/10min.

The conctractions, induced indirectly by electrical stimulation (frequency: 6 impulses/min) on frog *m. tibialis a. l.*, treated with CGE at a high concentration (25 µl/ml) showed in three preparations a gradual decrease in amplitude which reached zero at about 40 min. (Fig. 2A). Three similar preparations treated as above but stimulated only at intervals of 10 min, just to measure the contractions amplitude, (Fig. 2B) showed a similar pattern. Muscle contraction following direct stimulation was never impaired.

Three preparations treated with CGE at a concentration of 0.1 µl/ml, which is the lowest active concentration on *m. tibialis in vitro*, and stimulated indirectly at a frequency of 6/min, showed a retarded decrease in amplitude (Fig. 2C). The same behaviour was observed in three similar preparations stimulated only at intervals of 10 min (Fig. 2D). In all the above preparations the indirect response was still present, though reduced, 60 min after the application of CGE.

As shown in the graphs (Fig. 3A a, b) frog *m. tibialis a. l.* treated with CGE at a concentration of 25 µl/ml, undergoes a phase of contracture

lasting approximately as long as the fibrillation period (40-45 min). The duration of the contracture phase, however, decreases if the muscle is periodically stimulated at a frequency of 6/min (Fig. 3A c, d). Two preparations of mouse *m. rectus abdominis* treated with CGE at a concentration of 50 µl/ml showed a longer contracture lasting about 130 min (Fig. 3B).

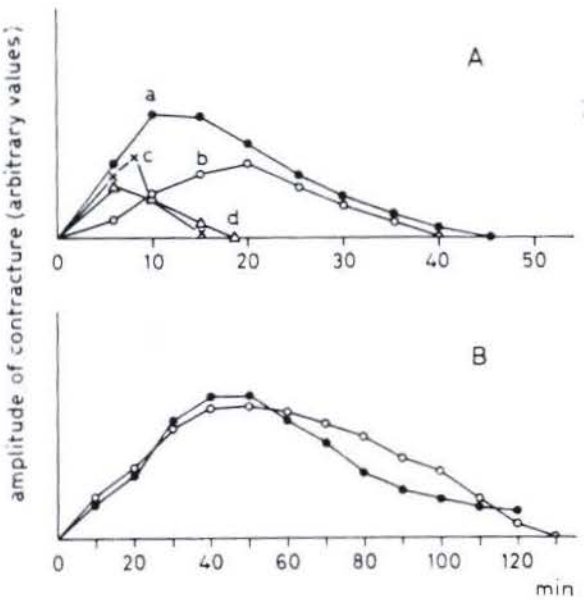



Fig. 3. — (A) trend of contracture phase of frog *m. tibialis anticus longus* following application of CGE (25 µl of conc. extract/ml): (a, b) no stimulation; (c, d) indirect stimulation at a frequency of 6/min. (B) trend of contracture phase of mouse *m. rectus abdominis* following application of CGE (25 µl of conc. extract/ml).

Application of CGE at a high concentration (50 µl/ml) did not cause any fibrillation, or contracture, in frog *m. tibialis a. l.* denervated 10-15 days earlier, or in curarized preparations. Only at extremely high concentrations, 75-100 µl/ml, a slight contracture was observed. Controls on denervated or curarized muscles showed, as expected, no response to indirect stimulation and normal response to direct stimulation.

Fig. 4 illustrates the effect of CGE, at a concentration of 25 µl/ml, on frog *m. tibialis a. l* after application of eserine ($1.3 \cdot 10^{-5}$ g /ml). The intensity and duration of fibrillations due to CGE are increased in preparations pretreated with eserine, as compared to the effects of CGE alone (Fig. 1A) where the preparation had been treated with higher concentration (50 µl/ml). Controls confirmed that eserine at this concentration does not cause any evident cynetic activity of the muscle.

The effect of venom + ACh on a curarized frog *m. tibialis a. l.* preparation is shown in Fig. 5. The preparation was pretreated with d-tubocurarine (15 µg/ml) for 10-15 min, after which the application of $10^{-5}$ ACh induced no contraction. Then CGE (50 µl of concentrated solution/ml saline) was

added. After 6 min, application of $10^{-5}$ g/ml of ACh brought about a response even larger in amplitude than that obtained with $10^{-4}$ g/ml of ACh before the curarized preparation had been treated with CGE.

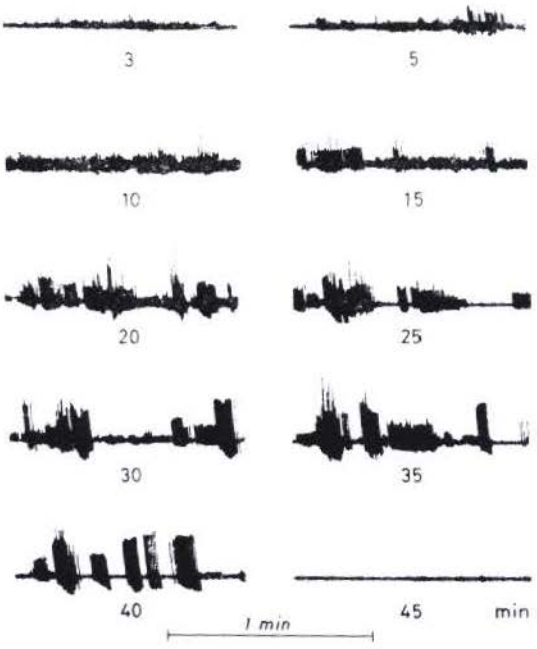
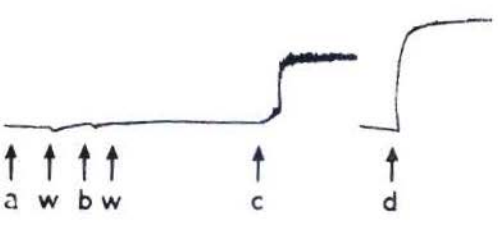



Fig. 4. — Motility of eserinized ($1.3 \times 10^{-5}$ mg/ml eesrine) frog *m. tibialis anticus longus* following application of CGE (25 µl of conc. extract/ml).

In another experiment the muscles *gastrocnemius*, *peronierus* and *tibialis a. l.* were dissected out from both limbs of a frog, treated for 10 min with CGE at a concentration of 50 µl/ml and, after washing, were kept for 60 min in saline containing $1.3\ 10^{-5}$ g/ml of eserine. The eserinized saline, placed

Fig. 5. — Effect of CGE and ACh on frog *m. tibialis anticus longus* following 10-15 min contact with d-tubocurarine (15 µg/ml): *a*, ACh $10^{-6}$ mg/ml; *w*, washing with saline; *b*, ACh $10^{-5}$; *c*, ACh $10^{-4}$; *d*, ACh $10^{-5}$ after 6 min contact with CGE (50 µl of conc. extract/ml).




then in contact with frog *m. abdominis*, caused the muscle to contract in a measure comparable to that obtained on the same preparation with $10^{-8}$ g/ml of ACh.

Antivenin (30 µl/ml), 10 min after application of CGE 1.6 µl/ml to *m. tibialis a. l.*, determined within 12 min the disappearance of both fibrillation and contracture.

Comparative studies on insect neuromuscular junction did not show any alteration of the indirectly induced contractions (frequency 6/min) nor

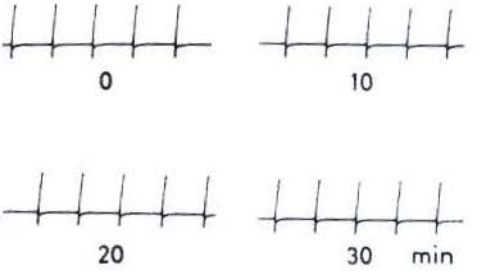



Fig. 6. — Application of CGE (50 µl of conc. extract/ml) on cockroach extensor muscles of the trochanter indirectly stimulated at a frequency of 6/min. No modifications of response were observed.

spontaneous fibrillations or contracture (Fig. 6), after incubation for 30-40 min in a solution containing 50 µl/ml of CGE.

## *Discussion*

On the basis of the results of our experiments, it is confirmed that CGE determines bursts of fibrillations in vertebrate muscle preparations. The phenomenon had already been observed by BELL & BOONE (1945) in a human case of latrodectism (« fine fibrillar twitchings of isolated muscle fibers were present, especially in the pectoral groups») and it was also noticed on frog muscle *in vitro* as early as 1929 by TROISE and recently by MAURO (LONGENECKER *et al.*, 1970). Furthermore it is proven that CGE at high concentrations causes a reduction to zero of the indirectly induced contractions, has no effect on the directly induced contractions and determines a contracture of the muscle preparation. The fact that muscle preparations, after the disappearance of contractions due to indirect stimulation, maintain their normal contractility to direct stimulation, confirms that the venom causes no impairement of the muscle fibres. The intensity and duration of the fibrillation and contractures phases appear to depend on the concentration of CGE. While the fibrillation phase is shorter in the mouse *m. rectus abdominis*, its contracture phase is about twice as long, at equal CGE concentrations, as that of frog *m. tibialis a. l.* The duration of both phenomena, as observed in different muscle preparations, may be related to the number of nerve terminals/muscle fiber. It should be recalled in this respect that latrodectism in humans determines a marked contracture of the adbominal wall muscles which usually disappears in a few hours after bite and only occasionally persists for 24 hours (BETTINI & CANTORE, 1959).

Our results seem to be in accordance with the mode of action characteristic of the BWS venom proposed by LONGENECKER *et al.* (1970) and CLARK *et al.* (1970). According to the hypothesis of these authors, the venom acts

on the nerve endings by determining a massive release of ACh quanta. The relatively high amount of ACh released apparently cannot be coped with by the AChE present at the synaptic junctions, which leads to ACh accumulation.

The mode of action of BWS venom on vertebrate neuromuscular junction seems, therefore, similar to that of β-bungarotoxin obtained from the snake *Bungarus multicinctus* venom. Here too the toxin acts presynaptically on the motor nerve endings, causing a transient increase, followed by complete disappearance, of miniature endplate potentials. Almost complete depletion of synaptic vescicles was observed electromicroscopically (LEE & CHEN I-LI, 1970).

The fact that the fibrillation and contracture start and come to an end simultaneously is in favour of the hypothesis that the two phenomena are correlated and possibly due to the same agent, in our case ACh.

As expected, therefore, CGE shows no effect, *i. e.* no fibrillation nor contracture, on the denervated muscle (*). In the case of preparations treated with curare, which acts by competing with ACh, CGE induces a massive release of transmitter which potentiates the effect of the ACh added to the bath. It should be expected that an increase of ACh at nerve terminals, induced by repetitive indirect stimulation of the muscle, would cause a more rapid effect by enhancing the depletion of ACh vescicles if paralleled by the action of CGE. This, however, was not the case even when the concentration of CGE employed was just sufficient to decrease the muscle response without causing complete block within one hour.

The reason for the non-appearance of this summation of effects lies probably in the fact that the amount of ACh released at each end-plate following nerve stimulation, *i. e.* about 30.000 quanta on a 50 min period [calculated on the basis of 100 q/stimulus (ENCELL, 1970)] is very low compared to that released throught the effect of CGE, *i. e.* about 500.000 quanta within a 40-50 min period (LONGENECKER *et al.*, 1970) and may not be appreciated in the recording.

Following the application of low concentrations of CGE (1,6 μl/ml) to frog *m. tibialis a. l.*, fibrillation follows a different pattern showing reduced frequency and intensity. The contraction amplitude is also decreased, but 90 min after application of CGE the preparation shows no signs of block. On the other hand, as demonstrated by LONGENECKER, *et al.* (1970) the frog *m. sartorius* miniature end-plate potentials reach zero level 75 min after the application of an equal concentration of CGE. This difference in

(*) SAMPAYO (1944) had observed that trembling, contractions, and stiffness were absent if muscles had been denervated and this observation led him to consider the venom as acting on the CNS.

behaviour of the two preparations is probably due to the fact that in Longenecker's experiments CGE had reached more readily the surface muscle fibers, *i. e.* those which are impaled by microelectrodes, while in our case the venom might not reach at all the inner fibers of the intact muscle bundle.

At this point we may strongly suspect that the muscle contracting substance, released in the eserinized fluid by isolated muscles previously treated with venom, is ACh itself. This is also supported by the recent findings of FRONTALI and PARISI (personal communication) who observed that rat brain cortex slices incubated with CGE release 100 % more ACh into the medium.

If a parallelism can be tentatively suggested between the mode of action of BWS venom on vertebrates neuromuscular preparations and the symptoms related to muscular impairment in man, the same cannot be said about the recovery of symptoms. *In vitro* experiments showed that at high CGE concentrations the muscle preparation is unable to recover except when treated, before exhaustion, with antivenin. At low concentrations, however, it was observed that the contraction amplitude decreases but never reaches zero. This may be due either to lack of penetration, since only the superficial fibers come in contact with the venom, or to a weaker toxic action of the diluted venom on all the neuromuscular junctions. Only the latter case applies to vertebrates bitten by the BWS, for in this case the venom reaches the muscle fibers through the blood and/or lymphatic vessels. Since BWS bite in humans is responsible for the delivery of a very low amount of venom per unit body weight, it should be assumed that a low concentration affects practically all neuromuscular junctions. It appears from the BWS bite symptomatology that the action of the venom persists in the majority of cases for 48-72 hours, but in rare cases pain and muscular weakness may well last three months or even more (ANON., 1937; BAERG, 1923; GAND & DALESALLE, 1949; MARETIC, 1951).

It should be recalled that at any stage of the syndrome, the administration of antivenin completely eliminates all symptoms. From this it appears that, unless neutralized by antivenin, the venom toxic proteins persist and remain active for a rather long period until aspecific proteinases metabolize them or spontaneously degenerate.

It cannot be excluded, however, that the toxic protein may cause an irriversible damage to the neuromuscular junction and that a slow « reinnervation » may take place as it occurs in incompletely denervated fibers (MILEDI, 1960).

It is not surprising that CGE shows no effect on the neuromuscular junctions of the cockroach motor muscles. Our results are in agreement with those of PARNAS & RUSSELL (1967) who found non evident effects following the application of *L. mactans* venom on the deep extensor abdomi-

nal muscle, medial, of the crayfish *Procambarus clarki*. The arthropod motor neuromuscular junctions have been proven to behave pharmacologically quite differently from the vertebrate junctions and to be definitely ACh-insensitive (KERKUT & WALKER, 1966).

*Note:* We read the recent paper by Okamoto *et al.* (*Science*, **172**, 733, 1971) only after the presentation of the present work. Our conclusions are on the whole in agreement with those of Okamoto *et al.* What is still open to discussion, however, is the fate of vertebrate nerve terminals after BWS bite *i.e.*, when the toxic syndrome (fibrillations, contractures, etc.) caused by the inoculation of a very small amount of venom is followed in the majority of cases by complete recovery within a 2-3 day period.

*Received* June 7, 1971.
*Accepted* July 27, 1971.

## REFERENCES

ANONIMUS, 1937. Black widow spider bite. *J. Am. Med. Assoc.*, **109**, 381.

BAERG, W. J., 1923. The effects of the bite of *Latrodectus mactans* Fabr. *J. Parasitol.*, **9**, 162.

BELL, J. E, & J. A. BOONE, 1945. Neostigmine methyl-sulfate an apparent specific for arachnidism (Black widow spider bite). *J. Am. Med. Assoc.*, **129**, 101.

BETTINI, S. & G. P. CANTORE, 1959. Quadro clinico del Latrodectismo. *Riv. Parassitol.*, **20**, 49.

CANTORE, G. P., 1958. Contributo allo studio dell'azione farmacologica del veleno di *L. tredicimguttatus* Rossi. *Riv. Parassitol.*, **19**, 158.

CLARK, A. W., A. MAURO, H. E., LONGENECKER Jr. & W. P. HURLBUT, 1970. Effects of BWS venom on the frog neuromuscular junction. Effects on the fine structure of the frog neuromuscular juncture. *Nature*, **225**, 703.

D'AJELLO, V., A. MAURO & S. BETTINI, 1969. Effect of the venom of the BWS *Latrodectus mactans tredecimguttatus*, on evoked action potential in the isolated nerve cord of *Periplaneta americana*. *Toxicon*, **7**, 139.

D'AMOUR, F. E., F. E. BECKER, & W. VAN RIPER, 1936. The Black Widow Spider. *Quart. Rev. Biol.*, **11**, 123.

ENCELL, B. J., 1970. Experiments on synaptic transmission using the frog sartorius, nerve muscle preparation. In: *Experiments in physiology and biochemistry*. G. A. Kerkut Ed., **3**, 211.

GAND, J. & D., DELESALLE, 1949. Aranéisme dû aux morsures de Latrodectes en Maroc. *Bull. Inst. Hyg. Maroc.*, **9**, 232.

KERKUT, G. A. & R. I. WALKER, 1966. The effect of L-glutamate, acetylcholine and gamma-aminobutyric acid on the miniature end-plate potentials and contractures of the cockroach *Periplaneta americana*. *Comp. Biochem. Physiol.*, **17**, 435.

LEE, C. Y. & I-LI. CHEN, 1970. Effect of β-bungarotoxin on synaptic vesicle. *Abstracts from 2nd Intern, Symp. on Animal and Plant Toxins*, Tel Aviv. p. 59

LONGENECKER, H. E. Jr., W. P. HURLBUT, A. MAURO & A. W. CLARK, 1970. Effects of BWS venom on the frog neuromuscular junction. Effects on end-plate potentials miniature end-plate potential and nerve terminal spike. *Nature*, **225**, 701.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR & R. J. RANDALL, 1951. Protein measurement with the folin-phenol reagent. *J. Biol. Chem.*, **193**, 265.

MAJORI, G., S. BETTINI & O. CASAGLIA. Effect of Black Widow Spider venom on the cockroach heart (in press.).

MARETIC, Z., 1951. Beobachtungen über Pathologie und Klinik des Latrodektismus in Istrien. *Acta Tropica*, **8**, 136.

MILEDI, R., 1960. The acetylcholine sensitivity of frog muscle fibers after complete or partial denervation. *J. Physiol.*, **151**, 1.

PARNAS, I. & F. E. RUSSELL, 1967. E ects of venoms on nerve, muscle and neuromuscular junction. *Animal Toxins*, Pergamon Press, Oxford & New York, p. 401.

RUSSELL, F. E. & T. E. LONG, 1961. Effects of venoms on neuromuscular transmission. *Myasthenia Gravis*, Viets H. R. Ed., **3**, 101.

RUSSELL, F. E., B. A. O'BRIEN & D. I. INABA, 1961. Venoms and neuromuscular transmission. *Symp. on Venomous and Poisonous Animals and Noxious Plants*: (B) *Snakes. Proc. 10th Pacific Sci. Congr.*, Honolulu, p. 455.

SAMPAYO, R. R. L., 1942. *« Latrodectus mactans » y latrodectismo. Estudio experimental y clinico.* El Ateneo, B. Aires.

SAMPAYO, R. R. L., 1944. Pharmacological action of the venom of *Latrodectus mactans* and other *Latrodectus* spiders. *J. Pharmacol. Expl. Therap.*, **80**, 309.

TROISE, E., 1928. Action pharmacodynamique du venin de *L. mactans. Compt. Rend. Soc. Biol.*, **99**, 1431.

TROISE, E., 1929. Action du venin de l'Araignée *Latrodectus mactans. Compt. Rend. Soc. Biol.*, **102**, 1097.