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THE EFFECTS OF CURARE IN THE COCKROACH

II. BLOCKAGE OF NERVE IMPULSES BY dTC

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INTRODUCTION

Although for many years insects were believed to be insensitive to curare (Harlow, 1958; Hopf, 1952; Krupp, Lendle & Stapenhorst, 1952; Roeder, 1948, 1953; Roeder & Weiant, 1950; Usherwood, 1963), the recent report of Larsen, Miller & Yamamoto (1966) has demonstrated that insects are, indeed, sensitive to curare. Subsequent investigations (McCann, 1966; Flattum, Friedman & Larsen, 1967; Friedman, 1967) have been unable to elucidate the site or mechanism of insect curarization. However, studies of leg contraction in the cockroach (Friedman & Carlson, 1969) led us to the conclusion that curare most probably causes failure of contraction by preventing conduction of action potentials in motor nerve fibres. We furthermore proposed that blockage by curare of action potential conduction is the mechanism of insect curarization. This report supports our proposal by presenting electrophysiological evidence which indicates that curare (d-tubocurarine, dTC) blocks the conduction of action potentials in nerve fibres of the cockroach, *Periplaneta americana*.

MATERIALS AND METHODS

A. Preparations studied

1. The giant-fibre preparation (Fig. 1) was used to assess the effects of various test solutions on the ability of giant fibres within the nerve cord to conduct action potentials. The cockroach was first decapitated and its legs and wings were removed. The animal was then pinned on to Plasticine, ventral surface uppermost. The sterna overlying the nerve cord in the abdominal segments, A1–A2 and A5–A6 were dissected away. The nerve cord connectives between A5 and A6 were freed and a pair of platinum stimulating electrodes was placed under them. The nerve cord was then cut posterior to A1, and the A1–A2 connectives were dissected free of peripheral connexions. The freed connectives were then picked up in a suction electrode. Test solutions were applied to the A5–A6 region of the preparation with a Pasteur pipette.

2. The cercus–cercal nerve preparation (Fig. 3) was used to assess the effect of curare on a sensory system. An animal was first decapitated and its legs and wings were removed. It was then pinned on to Plasticine, dorsal surface upwards. After exposing the nerve cord both cercal nerves were dissected of their attachments and severed from the sixth abdominal ganglion. The freed ends of the cercal nerves were picked

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up in suction electrodes. To introduce test solutions into this preparation the last six segments of the cerci were first removed. Small drops of test solutions on the needle tip of a microlitre syringe were then applied by touch to the cerci. The solutions were drawn into the cerci by capillary action. In each experiment one cercus received a known volume of curare solution while the contralateral cercus received an equal volume of saline. Curare solution was always applied to the cercus whose cercal nerve yielded the higher initial endogenous activity.

3. The cercus–nerve cord preparation (Fig. 4) was used to assess the post-synaptic effect of curare application to the cercus. The preparation was made by first decapitating the animal and removing its legs and wings. The animal was then pinned on to Plasticine, ventral surface uppermost. The sterna overlying abdominal segments A1–A2 were removed. The exposed nerve cord was freed and cut below A1. The nerve-cord connectives were then picked up in a suction electrode. Test solutions were introduced into the preparation in the same manner as used for the cercus–cercal nerve preparation described above. Activity in each cercus was induced by passing an air current over the cerci.

4. The cercal nerve–nerve cord preparation (Fig. 5) was used to assess the effect of curare when the drug was applied to the cercal nerve. The cockroach was decapitated and its legs and wings removed. The animal was then pinned on to Plasticine, ventral surface uppermost. The sterna overlying the A4–cerci area were removed. One cercal nerve and the A4–A5 nerve cord connectives were freed of their surroundings. A pair of platinum stimulating electrodes was placed under the cercal nerve. The nerve cord was cut below A4, and the A4–A5 connectives were picked up in a suction electrode. Test solutions were applied to the A6–cerci region of the preparation with the aid of a Pasteur pipette.

B. Electronic procedures

A Grass S-4 stimulator was used to generate stimuli. The stimuli were fed through a Grass stimulus-isolation unit and delivered to the preparation with platinum hook electrodes. Stimuli were always of the same duration (0·1 msec.) and frequency (0·5 pulses/sec.). The voltage varied between 1·5 and 6·0 volts, as indicated in the Results. Electrode polarity was reversed to obtain consecutive stimuli of alternate polarity. Nerve impulses were recorded with a suction electrode. The suction electrode was composed of fine polyethylene cannula tubing and silver wire 0·1 mm. in diameter. The solution used to relay impulses to the silver wire within the tubing was the saline used for the dissection of the preparation. Nerve impulses were amplified by a Grass P-9 pre-amplifier. The approximate amplification gain used in these experiments was 2400. The output was displayed on an oscilloscope (Tektronix 502A) and photographed with a Grass Kymograph camera.

C. Solutions administered

The saline solution used was Pringle's (1938) insect saline. The test solutions included saline containing 0·032 M d-tubocurarine, 0·033 M sucrose and 0·032 M aspartic acid respectively. All solutions were buffered with Tris buffer to pH 7·4.

RESULTS

A. Curare-induced blockage of conduction in giant fibres

Stimulation of the giant-fibre preparation with external electrodes evoked a compound action potential followed by smaller, trailing potentials (Figs. 1, 2). Threshold stimulating voltages for such preparations were found never to exceed 2·0 V. Preparations maintained in saline were observed for as long as 330 min. without any changes attributable to deterioration (not shown). However, the irrigation of giant-fibre preparations with a 0·032 M curare solution caused a gradual increase in the voltage needed to evoke a compound action potential. As shown in Fig. 1 D, 120 min.

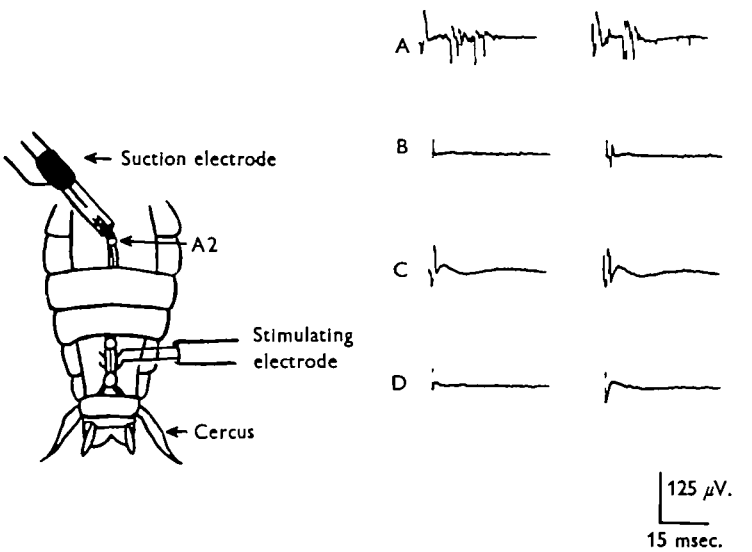

Fig. 1. Curare-induced blockage of conduction in giant axons. Application of a 0·032 M curare solution to a nerve cord causes a gradual increase in the voltage needed to initiate giant-fibre conduction. Two responses induced by stimuli of opposite polarity are shown on each line. A. Giant-fibre response 30 min. after saline irrigation of the nerve cord commenced. Stimulating voltage was 1·5 V. B. Giant-fibre response 30 min. after irrigation of the nerve cord with curare solution (0·032 M dTC). Stimulating voltage was 1·5 V. C. Giant-fibre response 30 min after irrigation of the nerve cord with curare solution (0·032 M dTC). Stimulating voltage was 3·0 V. D. Giant-fibre response 120 min. after irrigation of the nerve cord with curare solution (0·032 M dTC). Stimulating voltage was 6·0 V.

after the irrigation of a giant-fibre preparation with 0·032 M curare solution, a fourfold increase in the stimulating voltage failed to evoke an action potential. Failure to obtain a compound action potential with a stimulating voltage of 6·0 V. was taken to indicate blockage of conduction since it was initially observed that preparations which did not respond to stimulation at 6·0 V. would not respond to stimulation at higher voltages.

The conduction blockage seen after the application of 0·032 M curare solution to the giant-fibre preparation is due to the effect of the curare molecules *per se*. A number of experiments were undertaken to rule out other possible causes. Fig. 2 A, for example, demonstrates that the giant-fibre preparation maintains the ability to conduct impulses at threshold voltage 120 min. after initiating saline irrigation. The conduction blockage seen 120 min. after the application of curare solution cannot be due to the composition

of the saline solution. Conduction blockage is also not attributable to an osmotic imbalance caused by the addition of 0·032 moles of solute per l. of saline. As shown in Fig. 2B, the addition of 0·033 moles of sucrose per l. of saline does not produce conduction block. Finally, dTC is an acidic molecule, and an additional amount of Tris had to be added to hold the pH to 7·4. That this additional amount of Tris in the solution is not responsible for the conduction block was demonstrated by showing that a 0·032 M aspartic acid solution buffered with additional Tris does not cause conduction blockage (Fig. 2C). In contrast, 0·032 M curare solution produced conduction block even when the preparation was stimulated with a voltage of 4 × threshold (Fig. 2D).

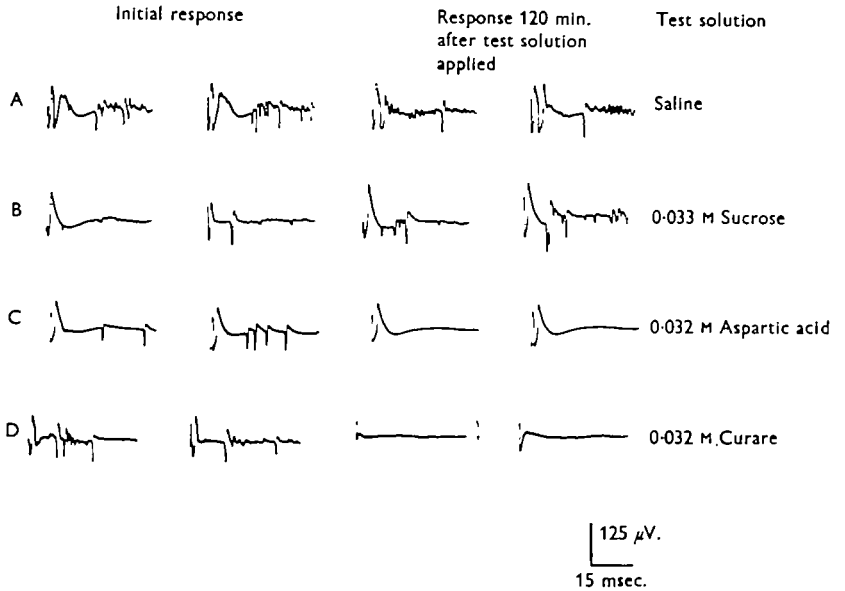

Fig. 2. Comparison of the effects of 'control' solutions and 0·032 M curare solution on giant-fibre conduction. The application of saline, 0·033 M sucrose or 0·032 M aspartic acid solution to the giant-fibre preparation does not induce conduction block. Two responses induced by stimuli of opposite polarity are shown on each line. A. Giant-fibre response to a stimulating voltage of 1·5 V. 60 and 120 min. after irrigation of the nerve cord with saline commenced. B. Giant-fibre response to a stimulating voltage of 1·5 V. before and 120 min. after irrigation of the nerve cord with 0·033 M sucrose solution commenced. C. Giant-fibre response to a stimulating voltage of 1·5 V. before and 120 min. after irrigation of the nerve cord with 0·032 M aspartic acid solution commenced. D. Giant-fibre response to a stimulating voltage of 1·5 V. before the application of curare solution, and the response to a stimulating voltage of 6·0 V. 120 min. after irrigation of the nerve cord with 0·032 M curare solution commenced.

B. Curare-induced blockage of sensory input

The cercal nerves of the cockroach conduct sensory impulses from the cerci to the sixth abdominal ganglion (A6). The extreme sensitivity of the cerci to air currents produces low-amplitude, high-frequency action potentials which can be recorded from the cercal nerves of an undisturbed cercus-cercal nerve preparation (Fig. 3A). The application and subsequent uptake by capillary action of 1·5 μl. of 0·032 M curare solution by a cercus blocked these action potentials. In contrast, the uptake of 1·5 μl. saline did not block such activity (Fig. 3B).

The cerci of the cockroach are connected via the cercal nerves and their synapses in A6 to the giant fibres of the nerve cord. Thus, passage of an air current over the region of the cerci increases the frequency of firing of the giant fibres. It is therefore possible to monitor the post-synaptic giant-fibre response to the application of curare to one cercus, and the response to the application of saline to the contralateral cercus. A diagram of the preparation used for such studies appears in Fig. 4. If the application

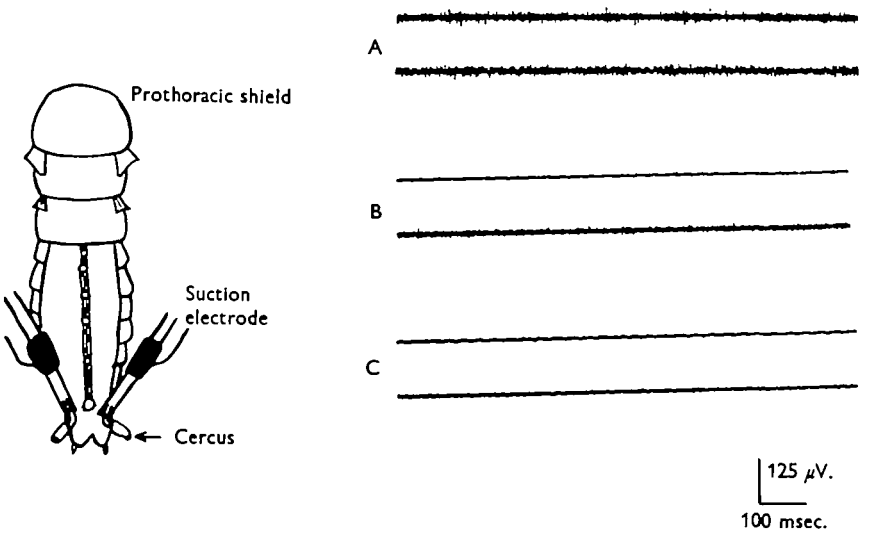

Fig. 3. Curare abolishes impulses in the cercal nerve. The application and subsequent uptake by a cercus of 1·5 μl. curare solution (0·032 M dTC) prevents the recording of nerve impulses from the cercal nerve. A. Activity of a cercus–cercal nerve preparation recorded 20 min. after its isolation. B. Activity of a cercus–cercal nerve preparation recorded 25 min. after 1·5 μl. curare solution was applied to one cercus and 1·5 μl. saline applied to the contralateral cercus. The upper trace monitors cercal nerve activity of the cercus treated with curare solution. The lower trace monitors cercal nerve activity of the cercus treated with saline. C. Noise level of the recording system monitored by removing the cerci from the preparation.

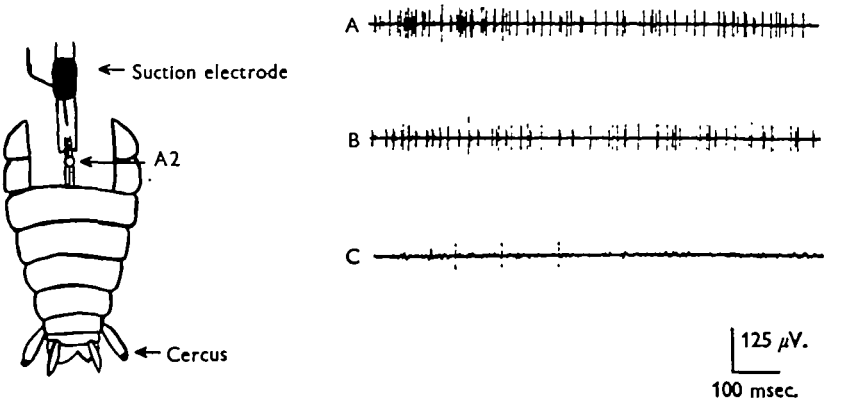

Fig. 4. Application of curare to a cercus prevents that cercus from contributing to the volley of giant action-potentials produced when an air current is passed over the cerci. A. Response 30 min. after 1·5 μl. of curare solution (0·032 M dTC) was drawn into one cercus, and 1·5 μl. of saline was drawn into the contralateral cercus of a cercus–nerve cord preparation. B. Response of the preparation after the removal of the curare-treated cercus. C. Response of the preparation after the removal of both cerci.

of curare to one cercus blocks impulses in its cercal nerve, then the giant-fibre response seen when the cerci are stimulated (by the passage of air) should be due to the cercal nerve input from the non-curarized cercus. Fig. 4 demonstrates that this was the case. The removal of the curare-treated cercus did not noticeably alter the giant-fibre response to the passage of an air current over the region of the cerci (compare Fig. 4B with Fig. 4A). The removal of the non-curarized cercus, however, prevented a giant-fibre response to the passage of an air current (Fig. 4C).

The application of 0·032 M curare solution to the cercal nerve–A6 region of the cockroach prevented the giant fibres from responding to electrical stimulation of the cercal nerve. As shown in Fig. 5A, the giant fibres responded to electrical stimulation of a cercal nerve with a burst of impulses. The giant-fibre response did not vary so

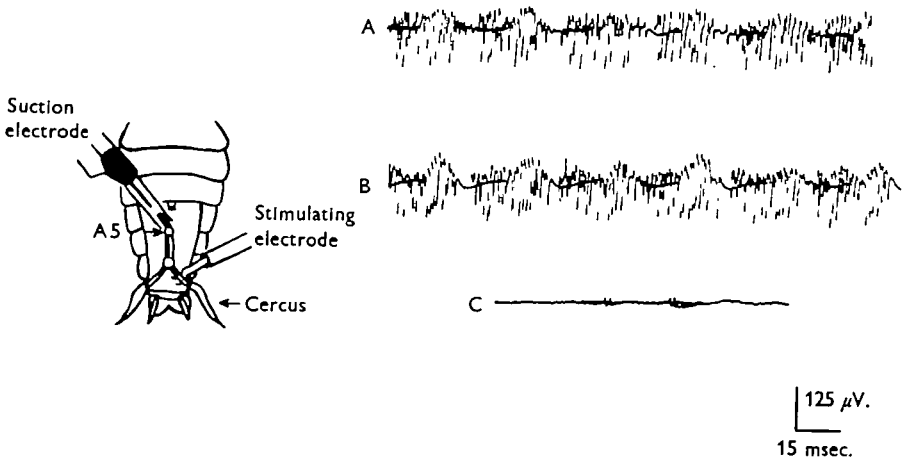

Fig. 5. Irrigation of the cercal nerve–A6 region of a cercal nerve–nerve cord preparation with curare solution (0·032 M dTC) prevents giant fibres from responding to electrical stimulation of the cercal nerve. A. Giant-fibre response to electrical stimulation of a cercal nerve (1·5 V.) 30 min. after irrigation of the preparation with saline. B. Giant-fibre response to cercal nerve stimulation (1·5 V.) 165 min. after irrigation of the cercal nerve–A6 region with saline. C. Giant-fibre response to cercal nerve stimulation 4× threshold (6·0 V.) 5 min. after irrigation of the cercal nerve–A6 region with curare solution (0·032 M dTC).

long as the cercal nerve–A6 region of the preparation was irrigated with saline. The substitution of 0·032 M curare solution for saline, however, prevented the giant-fibre response. Blockage of the giant-fibre impulses in response to electrical stimulation of the cercal nerve did not always occur within as short a time interval as occurred in the experiment shown in Fig. 5. Blockage did always occur within 120 min. of the application of 0·032 M curare solution.

DISCUSSION

In a previous study (Friedman & Carlson, 1969) evidence was given to suggest that curare blocks motor nerves in the cockroach. The results of this investigation demonstrate that it can block both sensory (cercal) and central (giant) axons. Giant fibres of the cockroach nerve cord fail to respond to electrical stimulation 120 min. after exposure to 0·032 M curare solution. Since the fibres are capable of conducting action potentials when exposed to 0·032 M aspartic acid solution, neither the presence of

extra Tris or the presence of a 0·032 M solution can be held responsible for the conduction block seen after the application of 0·032 M curare solution. Curare may act to block conduction in the cercal nerve because when a solution of this drug was applied to a cercus activity in the cercal nerve terminated. This possibility is further strengthened by the fact that application of dTC to the cercal nerve–A6 region of a cockroach blocks the post-synaptic giant-fibre response to cercal nerve stimulation.

Curare-induced blockage of action potentials has been previously reported for both vertebrate and invertebrate nerve fibres. Walsh & Deal (1959) described curare-induced blockage in the frog sciatic nerve, while Dettbarn (1960) described blockage of single nerve fibres of the same animal. Dettbarn (1963) achieved blockage of the walking leg nerve in the lobster, while Rosenberg and his colleagues have achieved conduction blockage in the giant axons of squid. All of these reports, however, involved some sort of pre-treatment of the fibre: exposure to venom and/or detergent, teasing out of individual fibres, or exposure to abnormal ionic conditions. In the current investigation fibres were not pre-treated in any manner. The studies which involve pre-treatment of nerve fibres to obtain a curare-induced blockage have been rightfully viewed with caution. However, the establishment of blockage without pre-treatment in this work leaves little doubt that curare can induce blockage.

If one accepts the blockage of conduction as a mechanism of curare action, some of the conflicts in previous reports concerned with the mechanism of insect curarization are resolvable. McCann (1966) was unable to inhibit muscle action potentials by directly applying dTC to the neuromuscular junction, yet inhibited these potentials by injecting curare. It seems reasonable that direct application of dTC to the neuromuscular junction was ineffective because dTC does not act at the insect neuromuscular junction. It also seems reasonable that dTC when injected intra-abdominally was effective because the drug was able to reach the motor axons and produce blockage.

Suga & Katsuki (1961) demonstrated that perfusion of the prothoracic ganglion of the grasshopper with 0·03% dTC blocked auditory impulses in the central nervous system. They argue that dTC penetrated the ganglion and acted on synapses inside the ganglion. This mechanism of action seems unlikely in view of the known inaccessibility of insect central nervous system synapses to drugs (Hoyle, 1965; Roeder, 1953) and the low concentration of dTC used. More probably, curare caused blockage in the tympanic nerve as it enters the prothoracic ganglion.

Friedman (1967) and Friedman & Carlson (1969) have shown that failure of leg contraction results when curare is injected into the abdomen, is injected into a leg or is applied to the metathorax of a cockroach. Mechanical studies revealed that the contraction failures observed in response to all three of these modes of curare administration are similar in nature. Thus, the contraction failures all seem to be due to one common mechanism of curare action. The one common mechanism is most probably the blockage by curare of action potential conduction in the motor nerve fibres.

The demonstration and proposal that curare acts in insects by blocking action-potential conduction in nerve axons seems correct for two other reasons: (1) it does not challenge the theory or the evidence which indicate that glutamate is the transmitter at insect excitatory neuromuscular junctions, and (2) it preserves the theory that curare acts at cholinergic neuromuscular junctions and at no other type of neuromuscular junction.

SUMMARY

1. The study of insect curarization in the cockroach, *Periplaneta americana*, has been continued. The application of curare solution (0·032 M dTC) to the nerve cord produced blockage of action-potential conduction in the giant fibres lying within the nerve cord.

2. The application of curare solution to the cerci prevented the recording of action potentials from the cercal nerves of the organism. Application of dTC to the cercal nerve–A6 region of the cockroach prevented giant fibres from responding to electrical stimulation of the cercal nerves. These results are interpreted as indicating that curare blocks the conduction of action potentials in the cercal nerve.

3. It is proposed that curare can induce blockage of conduction in sensory, motor and central nervous system fibres. It is further proposed that this blockage of conduction is the mechanism of insect curarization.

4. The results of previous reports concerned with insect curarization are re-interpreted in view of the proposal. Several of the conflicts in these reports are resolved by the proposal that blockage of conduction is the mechanism of insect curarization.

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REFERENCES

DETTBARN, W. D. (1960). Effect of curare on conduction in myelinated, isolated nerve fibres of the frog. *Nature, Lond.* **186**, 891–2.

DETTBARN, W. D. (1963). Action of acetylcholine and curare on lobster axons. *Life Sci.* **12**, 910–16.

FLATTUM, R. F., FRIEDMAN, S. & LARSEN, J. R. (1967). The effects of d-tubocurarine chloride on nervous activity and muscle contraction in the house cricket, *Acheta domesticus* (L.). *Life Sci.* **6**, 1–9.

FRIEDMAN, K. J. (1967). Peripheral and central effects of curare in the cockroach. *Am. Zool.* **7**, 734.

FRIEDMAN, K. J. (1969). The effects of curare on the central and peripheral nervous system of the cockroach, *Periplaneta americana*. Ph.D. dissertation. State University of New York, Stony Brook.

FRIEDMAN, K. J. & CARLSON, A. D. (1969). The effects of curare in the cockroach. I. dTC-induced failure of leg contraction. (in the Press).

HARLOW, D. A. (1958). The action of drugs on the nervous system of the locust (*Locusta migratoria*). *Ann. appl. Biol.* **46**, 55–73.

HOPF, H. S. (1952). Studies in the mode of action of insecticides. *Ann. appl. Biol.* **39**, 193–202.

HOYLE, G. (1965). Neural control of skeletal muscle. p. 408–450. In *Physiology of Insecta*. Ed. M. Rockstein. Vol. II. New York: Academic Press.

KRUPP, H., LENDLE, L. & STAPENHORST, K. (1952). Pharmakologische Wirkungen am isolierten Ganglien-Muskelpräparat des Gelbrandkäfers. *Arch. exp. Path. Pharmak.* **215**, 443–59.

LARSEN, J. R., MILLER, D. M. & YAMAMOTO, T. (1966). *d*-Tubocurarine chloride: effect on insects. *Science, N.Y.* **152**, 225–6.

MCCANN, F. V. (1966). Curare as a neuromuscular blocking agent in insects. *Science, N.Y.* **154**, 1023–4.

PRINGLE, J. W. S. (1938). Proprioception in insects. I. A new type of mechanical receptor from the palps of the cockroach. *J. Exp. Biol.* **15**, 101–13.

ROEDER, K. D. (1948). V. The effect of anticholinesterases and related substances on nervous activity of the cockroach. *Bull. Johns Hopkins Hosp.* **83**, 587–600.

ROEDER, K. D. (ed.) (1953). *Insect Physiology*. New York: John Wiley and Sons.

ROEDER, K. D. & WEIANT, E. A. (1950). The electrical and mechanical events of neuromuscular transmission in the cockroach, *Periplaneta americana*. *J. Exp. Biol.* **27**, 1–13.

ROSENBERG, P. (1965). Effects of venoms on the squid giant axon. *Toxicon* **3**, 125–31.

ROSENBERG, P. & EHRENPREIS, S. (1961*a*). Reversible block of axonal conduction by curare after treatment with cobra venom. *Biochem. Pharmac.* **8**, 192–206.

ROSENBERG, P. & EHRENPREIS, S. (1961*b*). Reversible block of axonal conduction by curare after treatment with cobra venom and a detergent. *Nature, Lond.* **190**, 728–9.

ROSENBERG, P. & HOSKIN, F. C. (1963). Demonstration of increased permeability as a factor in the effect of acetylcholine on the electrical activity of venom-treated axons. *J. Gen. Physiol.* **46**, 1065–73.

ROSENBERG, P. & HOSKIN, F. C. (1965). Penetration of acetylcholine into squid giant axons. *Biochem. Pharmac.* **14**, 1765–72.

ROSENBERG, P. & NG, K. Y. (1963). Factors in venom leading to block of axonal conduction by curare. *Biochim. Biophys. Acta* **75**, 116–28.

ROSENBERG, P. & PODLESKI, T. R. (1962). Block of conduction by acetylcholine and *d*-tubocurarine after treatment of squid axon with cottonmouth mocassin venom. *J. Pharmac. exp. Ther.* **137**, 249–62.

ROSENBERG, P. & PODLESKI, T. R. (1963). Ability of venoms to render squid axons sensitive to curare and acetylcholine. *Biochim. Biophys. Acta* **75**, 104–15.

SUGA, N. & KATSUKI, Y. (1961). Pharmacological studies on the auditory synapses in a grasshopper. *J. Exp. Biol.* **38**, 759–70.

USHERWOOD, P. N. R. (1963). Spontaneous miniature potentials from insect muscle fibres. *J. Physiol. (Lond.)* **169**, 149–60.

WALSH, R. R. & DEAL, S. E. (1959). Reversible conduction block produced by lipid soluble quaternary ammonium ions in cetyltrimethylammonium bromide—treated nerves. *Am. J. Physiol.* **197**, 547–50.