

SOME PRELIMINARY OBSERVATIONS ON THE EFFECTS OF CATIONS ON CONDUCTION PROCESSES IN THE ABDOMINAL NERVE CORD OF THE STICK INSECT, *CARAUSIUS MOROSUS*

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INTRODUCTION

The abnormal ionic composition of the haemolymph of some phytophagous insects is difficult to reconcile with some current concepts of the mechanism of nervous transmission. In contrast to the majority of arthropods and vertebrate animals which have been studied insects such as *Carausius morosus* possess a haemolymph in which the potassium level exceeds that of sodium, whilst magnesium ions are more concentrated than those of calcium in the haemolymph (Boné 1944; Duchâteau, Florkin & Leclercq 1953; Ramsay, 1953, 1955; Wood, 1957). Neuromuscular transmission in vertebrate animals (del Castillo & Engbaek, 1954) or in an insect such as *Locusta* (Hoyle, 1955) would rapidly become blocked at a fraction of the magnesium concentration in the haemolymph of *Carausius*. The nerve-muscle preparation of *Carausius* is also able to function effectively for extended periods in sodium-free solution and, in the apparent absence of an external diffusion barrier, it has been suggested that the magnesium ions may contribute to the muscle action current (Wood, 1957).

Nothing is known about the physiological mechanisms involved in the conduction processes of axons from insects possessing abnormal cation concentrations in the haemolymph. Studies on cockroach axons (Yamasaki & Narahashi, 1959) have demonstrated an essential similarity with transmission in cephalopod giant axons (cf. Hodgkin, 1951, 1958) and a dependence upon a high concentration of sodium ions in the haemolymph. The difficulties involved in considering nervous activity in insects with abnormal haemolymph could be resolved by the postulation of some sort of peripheral diffusion barrier (Hoyle, 1953; Twarog & Roeder, 1956). Some recent studies have shown, however, that the exchanges of various cations and non-electrolytes take place relatively rapidly across the sheath surrounding the cockroach central nervous system (Treherne, 1960, 1961*a, b*, 1962*a*). These appeared to be largely passive in nature and were believed to take place between the haemolymph and the extracellular spaces demonstrated by electron microscopy (Smith & Treherne, 1963). The present preliminary observations of *Carausius* were therefore undertaken in an attempt to elucidate some of the effects of cations on conduction in the central nervous system and, in particular, to investigate the effects of the presence of the nerve sheath on these processes.

METHODS

To measure the conduction processes in the abdominal nerve cord a small window was cut in the integument immediately above the connective joining the first pair of abdominal ganglia. The exposed connectives were then gently lifted on to a pair of fine silver wire electrodes. A similar procedure was carried out with the connectives between the fifth and sixth abdominal ganglia in order to place the silver wire recording electrodes in position. Attempts to reduce the drying of the preparation by covering the connectives with liquid paraffin, as in a previous investigation with *Periplaneta americana* (Treherne, 1962*b*) failed as this invariably resulted in a rapid loss of function in this insect. The exposed connectives and electrodes were therefore continually moistened with physiological saline and were carefully drained during the recording periods. This procedure resulted in more variability in results than was the case with the *Periplaneta* preparation. A third window was cut through the integument overlying the fourth abdominal ganglion and a small piece of nylon sheet was carefully drawn beneath the ganglion as described by Twarog & Roeder (1956) for *Periplaneta*. The exposed ganglion was irrigated with one or other of the various experimental solutions used in this investigation. In some experiments the ventral portion of the nerve sheath was removed from the ganglion by tearing with finely ground watchmaker's forceps as described by Twarog & Roeder (1956). The procedure used in the cockroach preparation, which was included for comparative purposes, was the same as in a previous investigation (Treherne, 1962*b*).

The nerve-cord preparation was stimulated by an uninterrupted series of rectangular pulses (0.5/sec.; 0.2 msec. duration) at low output impedance via an RF isolating unit. The recording system consisted of a differential preamplifier (Tektronix 122) coupled to a Tektronix type 532 oscilloscope.

The various physiological solutions used in these experiments were based on those for *Carausius* devised by Wood (1957). The saline approximating in ionic composition to normal haemolymph had the following composition: Na, 15; K, 18; Mg, 50; Ca, 7.5; H_2PO_4 , 6; HPO_4 , 4.5; Cl, 133 mM./l., together with 69.9 g./l. of trehalose. In the sodium-free solution potassium phosphates were substituted for sodium phosphates, the potassium chloride concentration being adjusted to maintain the normal level of the cation. Following Wood, phosphate-bicarbonate buffering (12 mM./l. H_2PO_4 ; 8 mM./l. HCO_3) was used for zero and 25.0 mM./l. magnesium salines to avoid the formation of phosphate precipitates at these low concentrations.

RESULTS

In satisfactory preparations conduction along the nerve cord remained relatively constant throughout the period of the experiment when the fourth abdominal ganglion was irrigated with the normal physiological solution resembling haemolymph in ionic composition (Fig. 1A). Normal conduction also persisted in this solution when the ventral portion of the nerve sheath of the fourth abdominal ganglion was torn away (Fig. 1B). By contrast, irrigation of the desheathed fourth abdominal ganglion of *Periplaneta* resulted in a rapid loss of conduction within a few minutes (Fig. 1C).

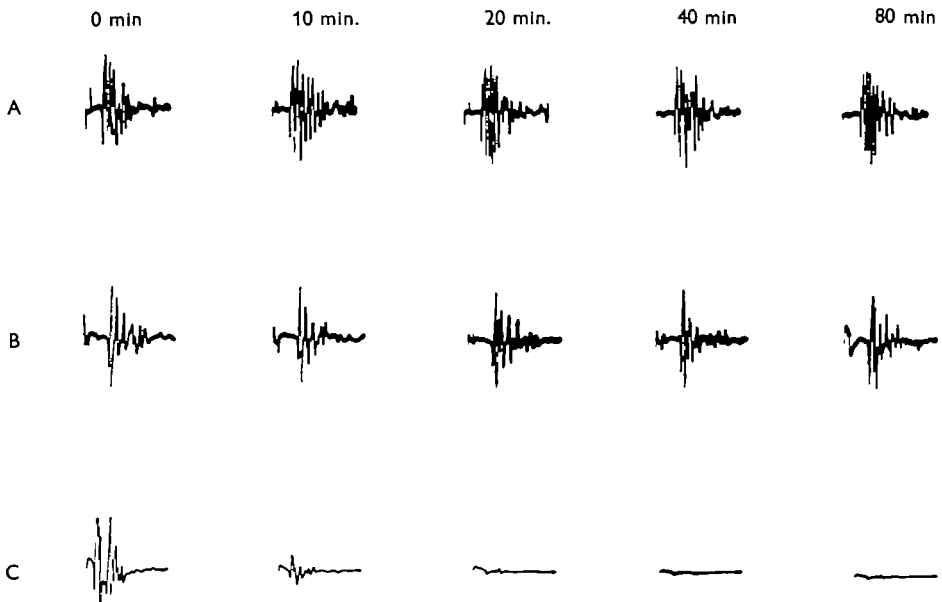


Fig. 1. Conduction in abdominal nerve cords of *Carausius* and *Periplaneta*. Complex action potentials obtained on irrigation of exposed fourth abdominal ganglion with saline resembling the haemolymph of *Carausius* in ionic composition. A, Intact ganglion of *Carausius*; B, desheathed ganglion of *Carausius*; C, desheathed ganglion of *Periplaneta*.

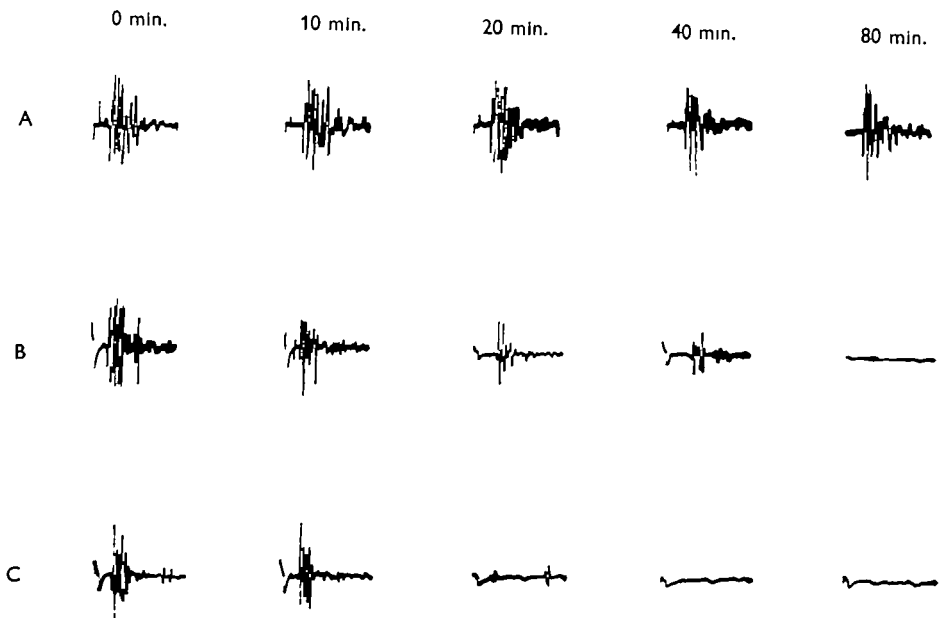


Fig. 2. Effects of sodium-free solution on conduction in abdominal nerve cord of *Carausius*. A, Intact fourth abdominal ganglion irrigated with normal solution; B, intact ganglion irrigated with sodium-free solution; C, desheathed ganglion irrigated with sodium-free solution.

Figure 2 illustrates the effects of sodium-free solutions on conduction processes in intact and desheathed fourth abdominal ganglia. In both cases the complex action potentials showed a progressive decline, a process which occurred more rapidly in the desheathed preparation.

Irrigation of the intact fourth abdominal ganglion with magnesium-free solution produced a relatively slow decline in conduction along the abdominal nerve cord (Fig. 3 A). Desheathed preparations irrigated with solutions of reduced magnesium concentrations (0 and 25.0 mM./l.) showed a more rapid loss of excitability (Fig. 3 B and C) than those irrigated with the solution containing the normal magnesium level of 50.0 mM./l. (Fig. 3 D).

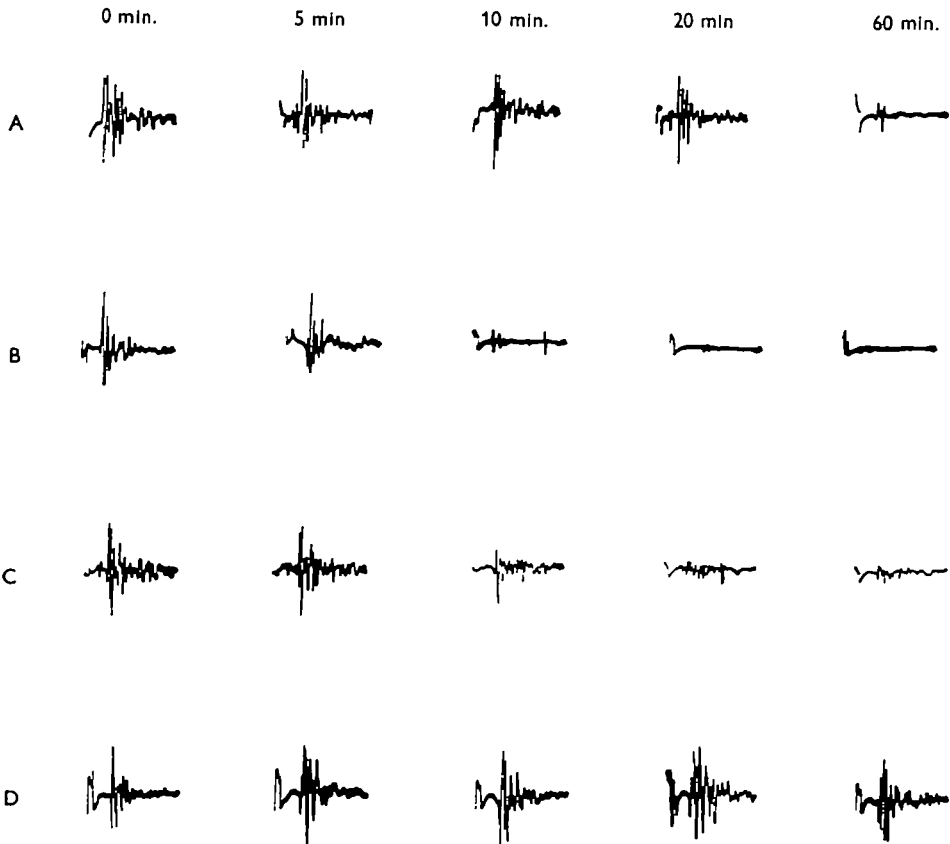


Fig. 3. Effects of magnesium concentration on conduction in the abdominal nerve cord of *Carausius*. A, Intact fourth abdominal ganglion irrigated with magnesium-free solution; B, desheathed ganglion irrigated with magnesium-free solution; C, desheathed ganglion irrigated with 25.0 mM./l. magnesium solution; D, desheathed ganglion irrigated with 50.0 mM./l. magnesium solution.

DISCUSSION

The results have shown that the conduction processes in the fourth abdominal ganglion continued when substantial portions of the nerve sheath were removed. Under similar conditions the complex action potentials of the cockroach nerve cord

showed a rapid decline when the preparation was bathed in *Carausius* saline. The ability of the axons of *Carausius* to function in a haemolymph with such an abnormal ionic balance does not, therefore, depend upon the presence of a peripheral diffusion barrier.

The present experiments have shown that sodium ions, despite their low concentration in the haemolymph, are essential to maintain transmission in the central nervous system of *Carausius*. The abdominal nerve cord apparently differs from peripheral nerve in this respect, for Wood (1957) has shown that a neuromuscular preparation (which included a portion of the crural nerve) could function for extended periods in sodium-free solution. These contrasting observations may well be the result of a difference in accessibility to these ions as between the central and the peripheral nervous systems in this insect. Such a differential permeability may also exist in other species, for the time for the development of complete conduction block in high-potassium saline was longer (by an order of magnitude) in the peripheral nerve of *Locusta* (Hoyle, 1955) than in the central nervous system of *Periplaneta* (Twarog & Roeder, 1956). Any restriction upon the entry of nutrient substances into peripheral nerves would not necessarily be a serious limitation, since the cell bodies of the neurones are contained within the ganglia, where from the present evidence and from data on the cockroach nerve cord (Treherne, 1960, 1961*a, c*, 1962*a*) there appears to be a ready exchange of ions and molecules with the haemolymph.

In addition to their dependence on the presence of sodium ions the axons in the central nervous system of *Carausius* were also demonstrated to be unable to function in the absence of external magnesium ions. Magnesium has also been shown to be essential in neuromuscular transmission in this insect (Woods, 1957) and it has been suggested that this ion may contribute to the inward current of the action potential. An influx of divalent ions is also thought to provide the action current in crab muscle fibres (Fatt & Katz, 1953; Fatt & Ginsborg, 1958). The possibility cannot, therefore, be eliminated that some similar mechanism may also be involved in the propagation of action potentials in the axons of the central nervous system of this insect.

SUMMARY

1. In the haemolymph of the stick insect *Carausius morosus* the concentration of potassium exceeds that of sodium and the concentration of magnesium exceeds that of calcium. The implications of this situation for nerve conduction have been studied.

2. Conduction is maintained in intact and desheathed preparations of the fourth abdominal ganglion under irrigation with a solution resembling haemolymph in ionic composition.

3. Action potentials recorded in response to electrical stimulation of the nerve cord decline in sodium-free solutions, both in intact and in desheathed preparations.

4. Conduction declines slowly under irrigation with magnesium-free solutions both in intact and in desheathed preparations.

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