

AXONAL FUNCTION AND IONIC REGULATION IN THE CENTRAL NERVOUS SYSTEM OF A PHYTOPHAGOUS INSECT (*CARAUSIUS MOROSUS*)

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INTRODUCTION

A limited number of invertebrate species, notably some freshwater molluscs and certain phytophagous insects, are known to possess body fluids whose composition is extremely specialized. In particular the sodium level may be very low in these species, attaining an extreme condition in the haemolymph of a coleopteran such as *Timarchia tenebriosa* in which the concentration of this cation amounts to only 1.6 mM./l. (Duchâteau, Florkin & Leclercq, 1953). It has been frequently remarked that such low sodium concentrations are difficult to reconcile with the conventional membrane theory for the propagation of the action potential, which depends upon the presence of a high concentration of this cation in the fluid bathing the axon surface.

Recent investigations carried out in this laboratory have been concerned with an attempt to elucidate the ionic basis of axonal conduction in the nerve cord of the stick insect, *Carausius morosus*, in which the sodium concentration of the haemolymph averages only about 15.0 mM./l., while the magnesium level is as high as 53 mM./l. (cf. Wood, 1957). Preliminary electrophysiological studies involving the use of extracellular electrode techniques indicated that the electrical conduction processes in an abdominal ganglion became sodium-dependent when the peripheral fibrous and cellular nerve sheath was removed (Treherne, 1965*a*). Radioisotope studies also demonstrated the existence of an appreciable rapidly-exchanging sodium fraction in the central nervous tissues of this species (Treherne, 1965*b*). This rapidly-exchanging fraction, which was identified as the extracellular sodium, did not appear to result from a simple Donnan equilibrium with the haemolymph, such as was demonstrated in the abdominal nerve cord of the cockroach (Treherne, 1962), but was found to be dependent upon an apparent active uptake of this cation from the haemolymph. More recently it was shown that the entire central nervous system is surrounded by a fat-body sheath in this species (Maddrell & Treherne, 1966). It was subsequently demonstrated that there was an appreciable potential of some 15-20 mV across the neural fat-body sheath, the interior being positive with respect to the bathing medium (Treherne & Maddrell, 1967). This positive potential appeared to result from a chloride diffusion potential across the fat-body sheath, although it did not appear to be directly associated with the maintenance of the relatively high concentration of sodium and chloride ions at the axon surfaces, for it was shown that the axons could function for extended periods in preparations from which the neural

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fat-body sheath was removed and which were bathed in low-sodium solutions. The ability of axons to conduct impulses in a nervous system bathed with low-sodium solutions thus appears to result most probably from the activity of the perineurial and/or glial cells in regulating the extra-axonal sodium level. The fine structure of the perineurium in the nerve cord of *Carausius* has been recently described by Maddrell & Treherne (1967). The present paper contains the results of experiments, using intracellular and extracellular electrodes together with electron microscope techniques, which were designed to throw some further light on the sodium-regulating system in the central nervous system of this insect species. An attempt has also been made to assess the role of potassium ions in the maintenance of the resting potential and that of magnesium ions in the production of the action potential in the axons in this species.

METHODS AND MATERIALS

The following procedure was adopted for fixation of lengths of the interganglionic connectives for examination with the electron microscope. A decapitated insect was cut open from the dorsal side and ice-cold fixative (2.5 % glutaraldehyde solution, buffered with cacodylate and containing 175 mM./l. sucrose) was poured on to the exposed ventral nerve cord. A length of the nerve cord was then removed and sliced into short lengths with a razor blade in a drop of ice-cold fixative on a wax block. The pieces were then transferred into fresh fixative and maintained at 0° C. for 3 hr. After washing for 24 hr. in several changes of cacodylate solution containing 350 mM./l. sucrose the pieces of nerve cord were post-fixed in cold veronal/acetate-buffered osmium tetroxide solution. Dehydration in an alcohol series then followed and, after replacement of the ethanol with propylene oxide, the tissue was embedded in Araldite. Thin sections were cut on glass knives using a Huxley ultramicrotome and, after double staining with uranyl acetate and lead citrate solutions, were examined using a Philips EM200 electron microscope.

In the majority of experiments the conduction processes were studied in the axons of the connectives between the pro- and meso-thoracic ganglia. The intracellular recordings were carried out on isolated nerve cords using a two-compartment nerve chamber. One compartment was a moist chamber and contained a pair of platinum wire stimulating electrodes. The connectives were passed through a small aperture, which was subsequently closed with a small Vaseline plug, into a second fluid-filled compartment. The connectives were maintained under tension by gripping them at the posterior end with watchmaker's forceps held in a micromanipulator. The region of the connectives to be impaled with the microelectrodes was supported by a pair of fine platinum wire hooks mounted on a micromanipulator.

The penetration of the relatively small axons (maximum size 6–8 μ) in the nerve cord of this species was found to be a matter of some difficulty. Penetration was facilitated by maintaining the connectives under an appropriate degree of tension and by inserting the microelectrodes at an angle of 45° to the connectives. In these experiments the tip of the microelectrode was slowly advanced into the tissue. When the tip encountered the axon surface a small monophasic spike of a few millivolts was recorded. Penetration of the axon was then effected by tapping the base of the micromanipulator, the entry into the cell being signalled by the development of the

resting potential. In the majority of cases the resting potentials showed a decline after a relatively short period, frequently of only a few seconds in duration. The longest period for which a steady resting potential was maintained was 20 min. This decline in the measured resting potential was presumed to result from damage to the axolemma of these very small axons caused by the microelectrode. In these experiments, therefore, the action potentials were photographed immediately on penetration of the axons, so as to minimize any effects of damage caused by the electrode tip.

The above descriptions refer to experiments carried out with connectives from which the neural fat-body sheath was removed. Attempts were also made to impale axons in connectives from which the underlying fibrous and cellular nerve sheath was removed. This desheathing was carried out using fine, electrolytically-sharpened tungsten needles. It was found, however, that removal of the nerve sheath made the impalement of the axons a virtual impossibility. No further success was obtained when desheathed connectives were treated with α -chymotrypsin (cf. Tobias, 1958), when varying degrees of tension were applied or when the angle of penetration of the electrode was reduced. The electrical activity of the axons in desheathed preparations was, therefore, monitored with extracellular electrodes. In these experiments the anterior portion of the connective was placed on platinum wire stimulating electrodes in a moist compartment and the posterior portion over recording electrodes in a similar moist compartment. The middle segment of the connective, containing the desheathed portion, was contained in a fluid-filled compartment. The small apertures, through which the connectives were passed between the compartments, were made water-tight by small plugs of Vaseline.

The effect of potassium ions on the resting potential was investigated on cell bodies from the mesothoracic ganglia. For this the ganglia were desheathed, as described above, and the exposed cell bodies were impaled with microelectrodes the operation being viewed at high magnification with a binocular microscope.

The nerve preparations were stimulated by uninterrupted series of rectangular pulses (1.0/sec; 0.2 msec. duration) at low output-impedance via an RF isolating unit. The glass microelectrodes were filled with 3.0 M-KCl and had a resistance of between 20 and 60 M Ω (measured in 3.0 M-KCl). These microelectrodes were used in conjunction with a high-impedance cathode follower of unit gain, coupled to a Tetrionix 532 oscilloscope.

The normal physiological solution used in these experiments approximated in composition to *Carausius* haemolymph (Wood, 1957) and had the following composition: Na 15 mm./l.; K 18 mm./l.; Ca 7.5 mm./l.; Mg 50 mm./l.; H₂PO₄ 6.0 mm./l.; HPO₄ 4.5 mm./l.; Cl 133 mm./l. The isotonicity of the solution was maintained by the addition of 63.3 g./l. sucrose. Variations in the ionic composition of the solution were accommodated by suitable alterations in the sucrose content.

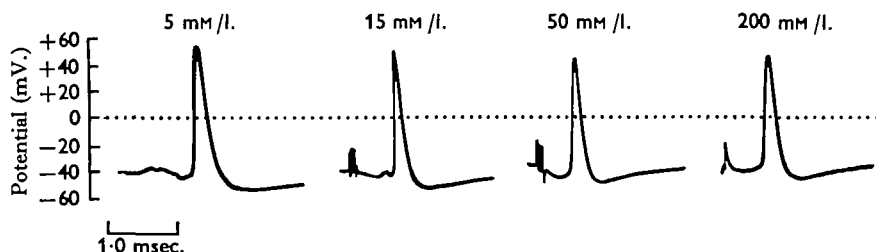
RESULTS

Axon-glial cell interrelations as revealed by the electron microscope

Of the tissues that separate the axons from the haemolymph the neural fat-body sheath and the perineurium have already been described (Maddrell & Treherne, 1966, 1967). The present report concerns the relations between the axons and the

surrounding glial cells. Plate 1 illustrates a typical field and shows several axons and their glial cell coatings cut transversely. The following points emerge from an examination of this and other similar electron micrographs;

- (i) Every axon is separated from the perineurium by at least one fold of glial cell.
- (ii) The glial cells are closely apposed to the surface of the axons, the extracellular cleft round the axons being only about 150 Å wide.
- (iii) In places the mesaxon (the intercellular cleft which connects the space immediately outside the axon surface with that beneath the perineurium), which is usually narrow, dilates considerably. These dilations are filled with a substance which is considerably more electron-opaque than either the axoplasm or the glial cell cytoplasm. Somewhat similar material occurs in similar dilations of the mesaxon in the cockroach nerve cord (Smith & Treherne, 1963).



Text-fig. 1. The effects of the sodium concentration of the bathing medium on intracellularly recorded action potentials in the intact thoracic connective. The records were obtained from preparations which had been exposed to the experimental solution for approximately 60 min. In this experiment, and in all subsequent ones, the neural fat-body sheath was removed prior to the introduction of the experimental solutions into the nerve chamber.

- (iv) The glial cell cytoplasm contains mitochondria which are similar in appearance to those occurring in the axoplasm and occur at about the same density. Also visible in the cytoplasm are fairly numerous microtubules.
- (v) At no point can desmosomes be seen holding the glial folds together.
- (vi) The mesaxons pursue a tortuous path so that the path from the axon surface to the space beneath the perineurium can be quite long.

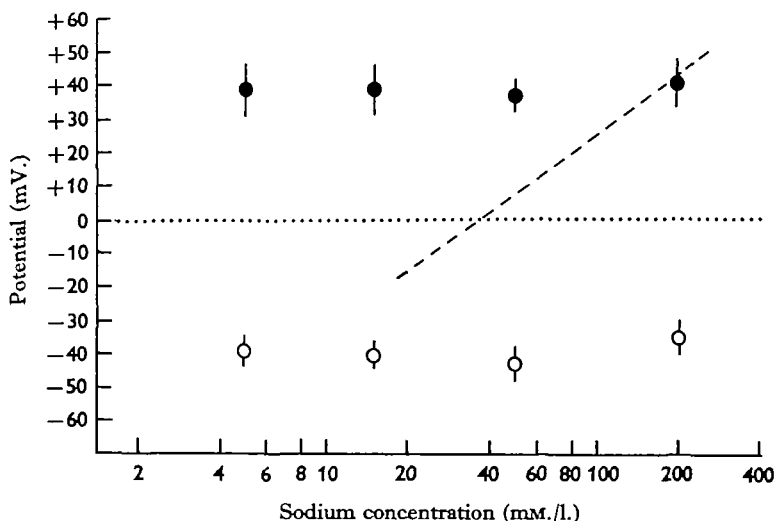
Effects of external sodium ions on resting and action potentials

The action potentials recorded in the axons of intact connectives, from which the neural fat-body was removed, were found to be unaffected by the sodium concentration of the bathing medium (Text-fig. 1). These action potentials were recorded from connectives which had been soaked in the particular physiological solution for periods of approximately 60 min. Both the resting potentials (which averaged 40 mV.) and the overshoot (39 mV.) remained relatively constant over the concentration range of 5–200 mM./l. NaCl (Text-fig. 2). It was also found that compound action potentials could be recorded for extended periods in preparations bathed with sodium-free solutions (Text-fig. 3).

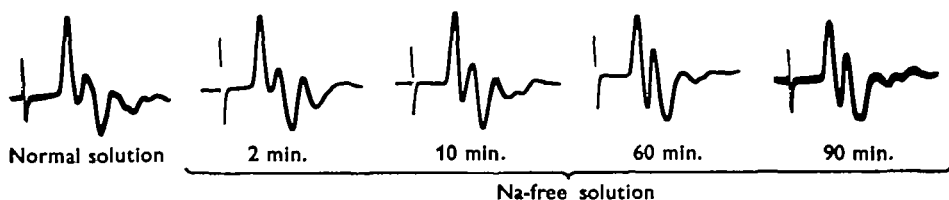
The above results contrast with those obtained with desheathed connectives, in which it was shown that treatment with sodium-free solution resulted in a rapid loss of conduction (Text-fig. 4). This effect could be readily and repeatedly reversed by treating the connectives with high-sodium solution (200 mM./l.).

Effects of tetrodotoxin on compound action potentials

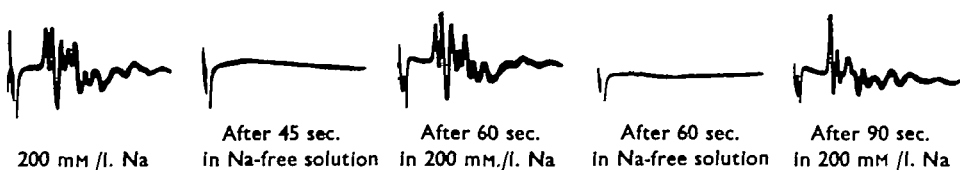
The treatment of connectives with dilute concentrations of tetrodotoxin resulted in a relatively rapid decline in the compound action potentials. This effect was achieved both with desheathed preparations and with those in which the nerve sheath was intact (Text-fig. 5). The decline in the amplitude of the compound action potentials did, however, occur more rapidly in the former preparations. These



Text-fig. 2. The relation between the sodium concentration of the bathing medium and resting potentials (open circles) and action potentials (closed circles) recorded from axons in intact preparations. The vertical lines drawn through the points indicate the extent of twice the standard error of the mean. The broken line illustrates the sodium equilibrium potential, calculated according to the Nernst equation from the data of Treherne (1965*b*).



Text-fig. 3. The effect of sodium-free solution on compound action potentials recorded in intact connectives.

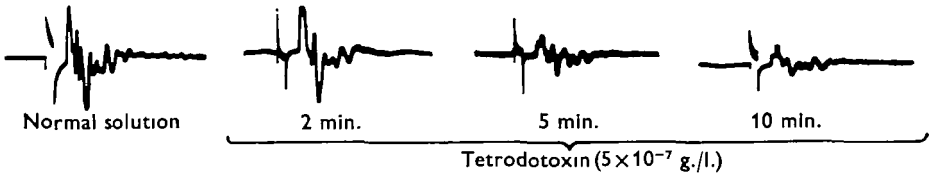


Text-fig. 4. The effects of sodium-free solution on compound action potentials recorded from desheathed thoracic connectives.

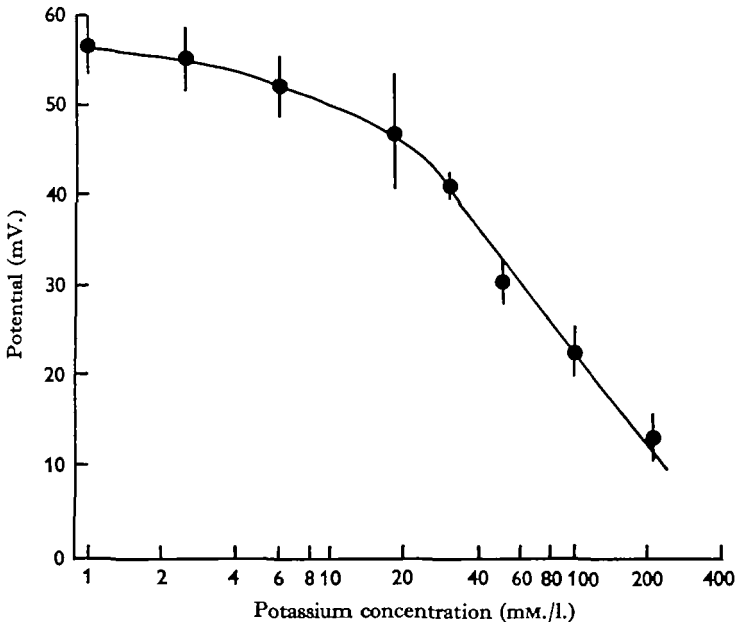
experiments were carried out using a solution containing an elevated sodium concentration, of 200 mM./l., so as to counteract any effects of the tetrodotoxin on the system involved in regulating the extra-axonal sodium level.

Effects of potassium ions on resting and action potentials

The resting potentials of impaled cell bodies in desheathed mesothoracic ganglia were somewhat greater than those recorded in the axons of intact connectives, averaging 46.5 ± 4.2 mV. in preparations bathed with normal physiological solution.



Text-fig. 5. The effect of externally applied tetrodotoxin on the compound action potentials recorded from intact thoracic connectives.



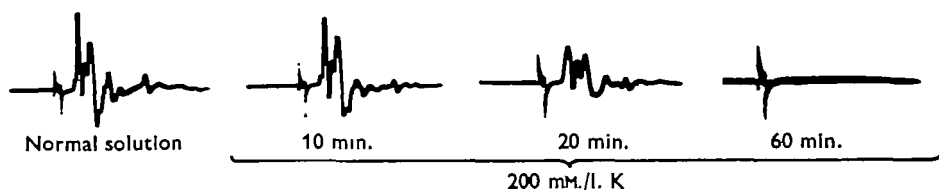
Text-fig. 6. The relation between the resting potential and the concentration of potassium in the bathing medium in cell bodies of desheathed mesothoracic ganglia.

Text-fig. 6 illustrates the relation between the resting potentials of cell bodies and the potassium concentration of the bathing medium. It will be seen that reduction of the external potassium level, from the normal concentration of 18.0 mM./l., had little effect on the resting potential, whereas increased potassium concentration resulted in a marked depolarization. The slope of the exponential portion of the graph, illustrated in Text-fig. 6, is approximately 37 mV. for a tenfold change in external potassium concentration.

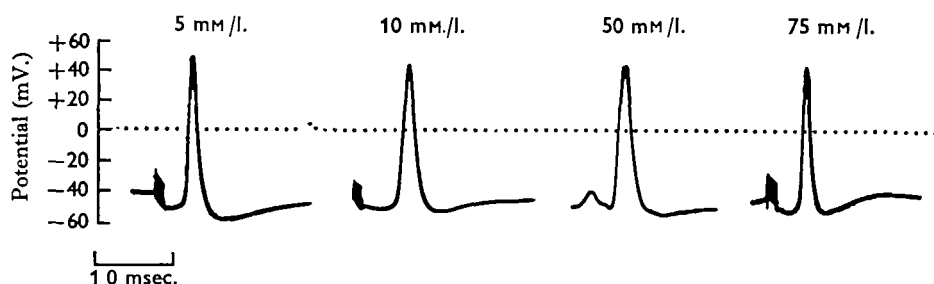
It is relevant to mention that the cell bodies impaled in these experiments did not

appear to be invaded by action potentials, for none were observed despite the varying methods of stimulation applied to the preparation. These insect cell bodies appear to be similar in this respect to those of crustacean ganglia (Preston & Kennedy, 1960).

Experiments were also carried out, using extracellular electrode techniques, in an attempt to determine the accessibility of potassium ions to the axon surfaces in intact preparations. In these experiments the compound action potentials were monitored in connectives which were exposed to an elevated potassium concentration of 200 mM./l. in the bathing medium. It will be seen, from Text-fig. 7, that there was a relatively slow decline in the recorded action potentials with the development of a complete conduction block.



Text-fig. 7. The effect of 200 mM./l. potassium in the bathing medium on compound action potentials recorded in intact thoracic connectives.

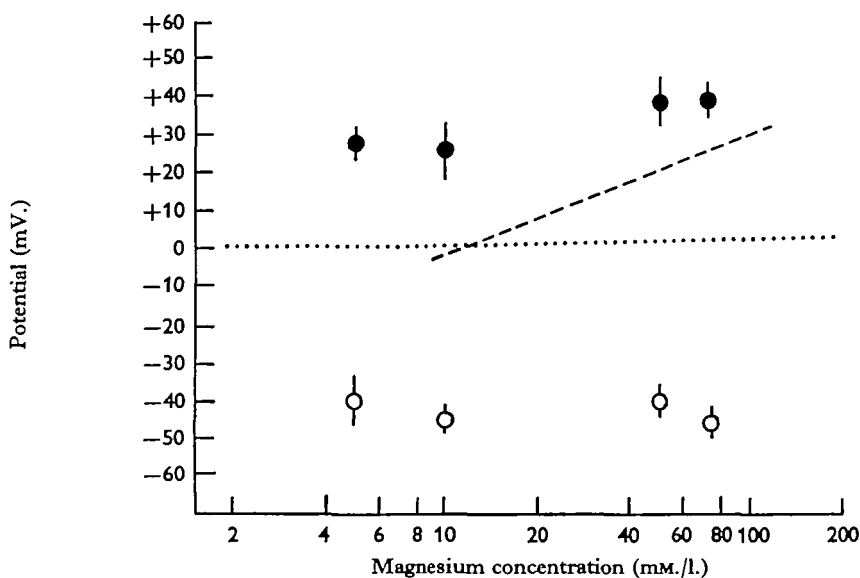


Text-fig. 8. The effects of the magnesium concentration of the bathing medium on intracellularly recorded action potentials in intact thoracic connectives. The records were obtained from preparations which had been exposed to the experimental solutions for a period of approximately 60 min.

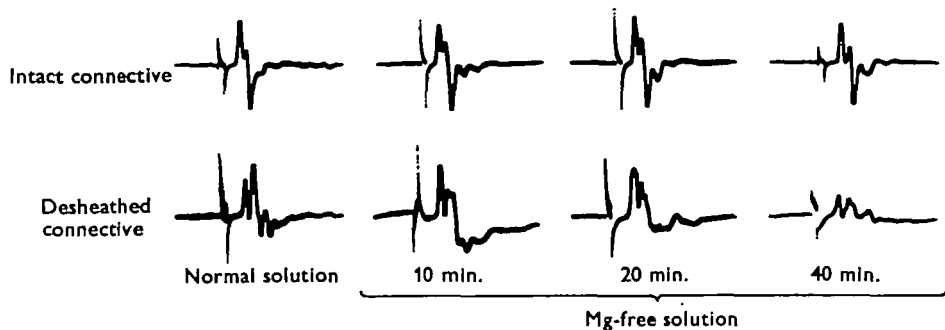
Effects of magnesium ions on resting and action potentials

Action potentials were recorded, with intracellular electrodes, in intact connectives bathed in solutions of varying magnesium concentration (Text-fig. 8). These results showed that very little effect on the resting and action potentials was produced by the variations in the external magnesium concentration (Text-fig. 9).

Experiments were also carried out to investigate the effects of magnesium ions on compound action potentials, monitored with extracellular electrodes. It was found that axons continued to conduct action potentials for extended periods in intact connectives bathed with magnesium-free solution (Text-fig. 10). Removal of the fibrous and cellular nerve sheath did, however, cause a relatively slow decline in the compound action potentials.



Text-fig. 9. The relation between the magnesium concentration of the bathing medium and resting potentials (open circles) and action potentials (closed circles) in intact thoracic connectives. The vertical lines drawn through the points indicate the extent of twice the standard error of the mean. The broken line illustrates the magnesium equilibrium potential calculated according to the Nernst equation from the data of Treherne (1965*b*).



Text-fig. 10. The effects of magnesium-free solution on the compound action potentials recorded from intact and desheathed thoracic connectives.

DISCUSSION

The ionic basis for the resting potential in the neurones of *Carausius* appears to be similar in some respects to that of *Periplaneta* giant axons. Thus in both species increasing the external potassium level results in depolarization. However, as in the cockroach (Yamasaki & Narahashi, 1959), the exponential slope obtained on plotting potassium concentration against resting potential does not show the 58 mV. change, for tenfold change in concentration, predicted by the Nernst equation. It is therefore clear that in *Carausius* neurones the resting potential is not solely determined by the potassium equilibrium potential and that the conductances to other ions must also contribute to the generation of the resting potential.

The above experiments were carried out on preparations from which both the neural fat-body sheath and the nerve sheath were removed so as to allow relatively unrestricted access to the neuronal surfaces. It has been found in a previous investigation (Treherne & Maddrell, 1967) that the resting potential in intact preparations was reduced by the interpolation of a positive potential of some 15 mV. between the haemolymph and the axon anterior. There was some evidence that this positive potential was associated with the neural fat-body sheath, for it was abolished in preparations in which this latter structure was removed. A similar positive potential, of about 15 mV., has also recently been observed in axons, in intact cockroach nerve cords, which tends to reduce the apparent resting potential (Pichon & Boistel, 1966). This latter species does not possess a complete neural fat-body sheath, which might suggest that its origin may differ in these two insect species. The possibility should be borne in mind, however, that some damage might have been caused to the neural lamella and/or perineurium in removing the fat-body sheath from the nerve cord of *Carausius*. Such damage would, of course, suggest a similar origin for this positive potential in the nerve cords of these two species. However, the fact that the connectives from which the fat-body sheath was removed continued to function for extended periods in sodium-free solution suggests that the structural integrity of the tissues was maintained, for desheathed nerve cords of *Carausius* have been shown to develop a rapid conduction block in the absence of external sodium ions.

The above results confirm those obtained in a preliminary investigation on ganglionic transmission in this insect (Treherne, 1965*a*). As in the previous study it was shown that axonal conduction in the connectives became sodium-dependent when the neural fat-body sheath and the underlying nerve sheath were removed. Under these conditions there was a rapid decline in the compound action potentials in low-sodium media and an equivalent rapid increase in spike height on exposure to solutions of high sodium concentration. It was also shown that a conduction block developed in the presence of dilute tetrodotoxin in both desheathed and intact preparations. This substance is thought to affect the sodium conductance channels involved in the production of action potentials in axons from other species (cf. Narahashi, Moore & Scott, 1964) so that the demonstration of its effect in the central nervous system of *Carausius* lends support to the concept that the action potentials in this species result from conventional mechanisms, (i.e. an influx of sodium ions from relatively high concentrations of this ion at the axon surfaces).

The present study has not revealed any significant evidence for the involvement of magnesium ions in the production of action potentials such as has been suggested as a possibility in this species (Treherne, 1965*b*). It was shown, for example, that there was a rapid decline in the amplitude of action potentials in desheathed preparations in sodium-free solution, despite the fact that the concentration of magnesium ions was maintained at its normal level in the haemolymph. Furthermore, there was only a relatively slow decline in conduction in desheathed connectives kept in magnesium-free solutions containing elevated sodium concentrations. These results would accord with the second possibility advanced in an earlier paper (Treherne, 1965*b*), namely that magnesium ions may be involved in the maintenance of the membrane structure necessary for the operation of the sodium conductance channels in the production of the action potential. This supposition can be compared with

the state of affairs in lobster giant axons, where the magnesium level of the bathing medium has been shown to affect action potential production (Dalton, 1959).

The demonstration of the sodium dependence of the axons in the central nervous system of *Carausius* raises the question of the precise mechanisms involved in the regulation of the extra-axonal sodium concentration. The efficiency of this mechanism has been adequately emphasized by the above experiments, which demonstrated the impressive ability of the axons to conduct action potentials for extended periods when the intact connectives were bathed with sodium-free solutions. There appear to be at least five fairly obvious methods by which the sodium level could be regulated at the axon surfaces in the presence of low concentrations of this cation in the haemolymph. These may be summarized under the following headings:

(1) *Regulation by the neural fat-body sheath*

It has recently been shown (Treherne & Maddrell, 1967) that there is a positive potential, of some 15 mV., between the extracellular fluid and the haemolymph in the nerve cord of *Carausius*. There is some evidence that this potential may be associated with the neural fat-body sheath which surrounds the central nervous system in this species (Maddrell & Treherne, 1966). This effect appears to involve a chloride diffusion potential, which, it is postulated, could result from the maintenance of a relatively high concentration of sodium and chloride ions at the inner surface of the fat-body sheath (Treherne & Maddrell, 1967). It was suggested that such a high concentration of sodium and chloride ions might be a consequence of the normal extrusion of these ions, that one supposes to occur in most cells, being directed on this side of the sheath into the restricted space enclosed between the neural lamella and the inner surface of the fat-body sheath. It seems reasonable to suppose that such extra-neural sodium would contribute to the relatively large rapidly exchanging sodium fraction demonstrated in the nerve cord of this insect (Treherne, 1965*b*). The present investigation has, however, demonstrated that the central axons continue to function for relatively long periods in the complete absence of the neural fat-body sheath in connectives bathed with low-sodium media. It appears, therefore, that the superficial fat-body sheath cannot be exclusively involved in regulating the extracellular sodium level in the central nervous system of this insect and it follows that some additional regulatory mechanisms must exist within the central nervous tissues.

(2) *Regulation by the neural lamella*

It might be assumed that the ability of axons to function in a nervous system bathed with a low-sodium solution might be a consequence of a restriction of cation movements by the fibrous layer of the nerve sheath. This hypothesis might be held to receive support from the fact that sodium regulation is effectively abolished in desheathed preparations. Experiments with radioactive isotopes have shown, however, that there is a rapid exchange of sodium ions between the central nervous tissues and the haemolymph in both *Carausius* (Treherne, 1965*b*) and *Periplaneta* (Treherne, 1961*a-d*, 1962). The effects of the desheathing procedure can also be reasonably attributed to damage caused to the underlying glial and perineurial cells, as well as to a complex series of drastic secondary alterations to the extracellular environment (Treherne, 1962).

(3) *Regulation by the perineurium*

The cellular layer of the nerve sheath has been regarded as the possible site of the sodium-regulating mechanism (cf. Shaw & Stobbs, 1963). This assumption would imply that the concentration of sodium ions in the entire extracellular system is maintained at a relatively high level by an inward secretion of these cations by the superficial cellular layer. There are, however, several observations which militate against this hypothesis. It has been shown, for example, that the axons could function for appreciable periods in connectives in which the surface was bathed with sodium-free solution. It is difficult to see how a high extracellular level of sodium ions could be maintained by an ion-pump, situated in this superficial cellular layer, in the absence of external sodium in the bathing medium. It is relevant to mention in this respect that the central axons of the cockroach can also function for relatively long periods in nerve cords bathed with low-sodium media (Twarog & Roeder, 1956). In this species there is no apparent regulation of the total extracellular sodium level, such as might be expected in this type of perineurial involvement, for the rapidly-exchanging (extracellular) sodium fraction was found to be related to the sodium concentration of the bathing medium (Treherne, 1962). The ultrastructure of the perineurial cells in *Carausius* does not appear to be obviously that which would be expected in an epithelium involved in a massive inwardly directed ion secretion (Maddrell & Treherne, 1967). The organization of the perineurium in this species is, in fact, strikingly similar to that of many fluid-secreting epithelia and it has been tentatively suggested that this cellular layer may be involved in the removal of water from the nerve-cord tissues.

(4) *Regulation by an extracellular fixed-charge system*

A previous investigation has shown that the large extracellular spaces in cockroach ganglia contain an acid mucopolysaccharide material (Ashhurst, 1961). Material of equivalent electron-density has also been shown to be present in the restricted extracellular spaces in the region of the axon surfaces and in the mesaxon folds (Smith & Treherne, 1963). The present study has also demonstrated the presence of an electron-dense substance in the dilation of the mesaxon clefts in the central nervous tissues of *Carausius* (Pl. 1). It has been suggested that the elevated concentrations of the extracellular cations in the cockroach nerve cord result from a Donnan equilibrium produced by the fixed anion groups associated with the acid mucopolysaccharide (Treherne, 1962). This system could thus function to maintain a relatively high concentration of sodium ions in the extracellular fluid. However, it is clear that the thermodynamic activity of the sodium ions in such a fixed-charge system would only be equivalent to that in the bathing medium and would not, therefore, contribute to the maintenance of an effectively high concentration of sodium at the axon surfaces. Any sodium ions associated with extracellular fixed anion groups would, however, tend to act as a cation reservoir. Thus it would be possible to visualize that any decrease in sodium concentration in the vicinity of the axons would be compensated for by cations associated with the fixed anion groups.

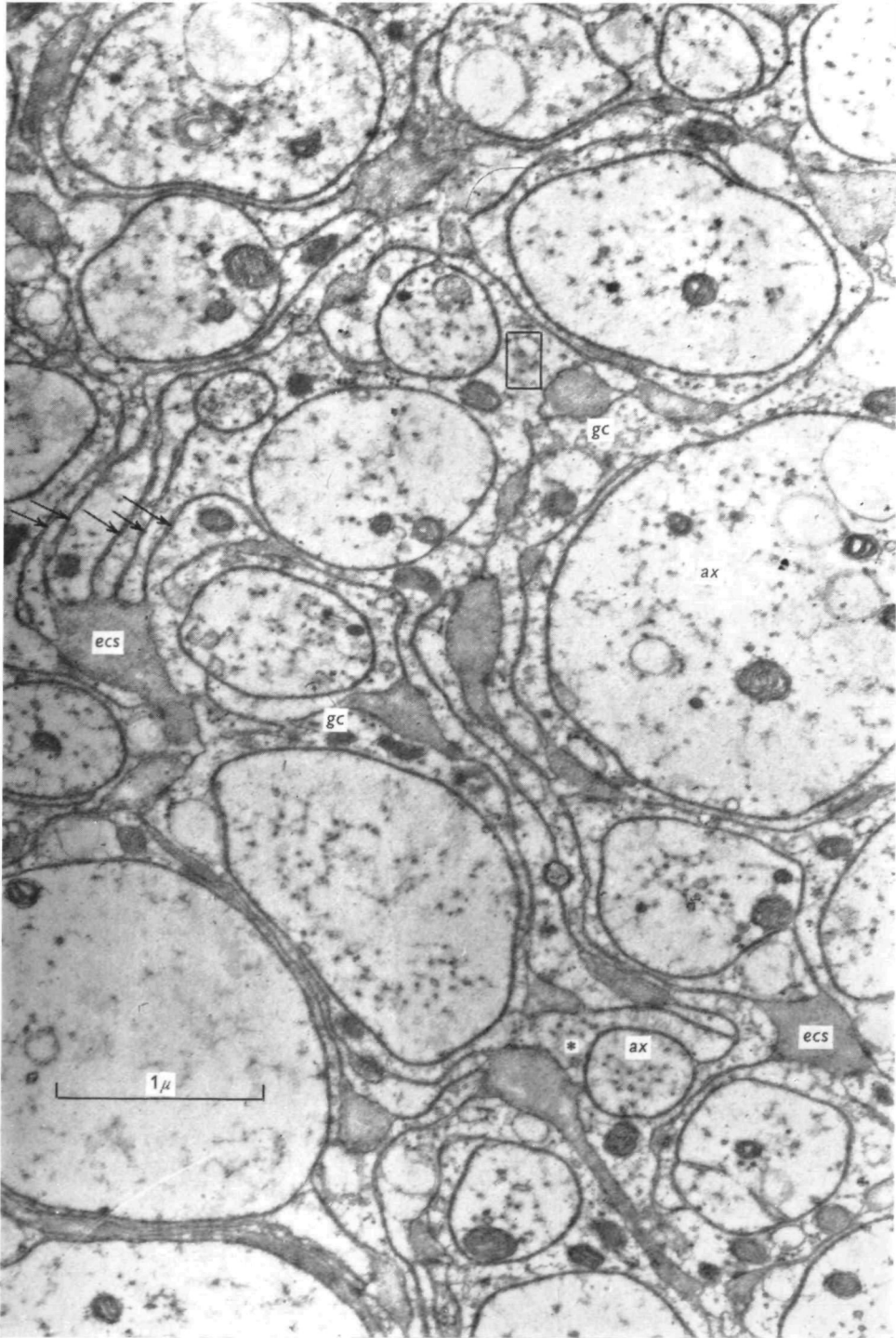
(5) *Regulation by the glial cells*

The available evidence indicated that in both *Periplaneta* (Twarog & Roeder, 1956) and *Carausius* axonal conduction continues for extended periods in the absence of sodium ions in the bathing medium. In both species, also, there appears to be a rapid exchange of this cation with the tissues of the central nervous system (Treherne, 1961*a-d*; 1962, 1965*b*). In the cockroach there is, in addition, no evidence of any significant regulation of the rapidly exchanging sodium, which has been identified with the extracellular ion fraction (Treherne, 1962). These observations suggest that the maintenance of the relatively high extra-axonal sodium level might be achieved not by a regulation of the general extracellular concentration of this cation but by local re-cycling of sodium in the region of the axon surfaces. The extrusion of sodium into the very restricted spaces adjacent to the axon surfaces (Pl. 1) could, for example, be conceived as producing a local high-sodium environment for the central axons. It is also conceivable that such a mechanism might be enhanced by a rapid *intracellular* movement, through the glial cytoplasm, of sodium ions, originating from the perineurial cells. This conception does not necessarily involve the postulation of any specialized sodium pumping mechanism, but only the conventional one known to be associated with the plasma membranes of the majority of cells which have been investigated. It is relevant to mention in this context that sodium extrusion from the cells in the nerve cords of these insects appears to be achieved by a single ouabain-sensitive carrier mechanism (Treherne, 1966).

The results obtained for *Carausius* contrast with the observations made on the leech central nervous tissues (Nicholls & Kuffler, 1964). In the annelid preparation it was found that alteration of the ionic composition in the bathing medium resulted in rapid changes in the membrane potentials of the nerve cells. With sodium-free solutions, for example, it was found that approximately 50% of the extra-axonal sodium was exchanged in less than 12 sec. The present investigation indicates that the differences in the physiological organization of the central nervous tissues in these two invertebrate groups result not from any significant exclusion of inorganic ions in the insect preparations, but from a dynamic regulation of the inorganic ions in the fluid bathing the axon surfaces.

SUMMARY

1. Experiments using intracellular and extracellular recording techniques indicate that, despite the specialized ionic composition of the haemolymph, the axons in the nerve cord of *Carausius* are conventional in that the action current is largely carried by sodium ions.
2. This effect is achieved by an appreciable regulation of the concentrations of inorganic ions in the extracellular fluid bathing the axon surfaces.
3. The extra-axonal regulation does not appear to result from any significant restriction in the accessibility of cations to the general extracellular system, but from a local regulation which appears to maintain a relatively high concentration of sodium ions at the axon surfaces.
4. It is suggested that such a regulation may be achieved by an extrusion of sodium



ions from the glial cells into the restricted extra-axonal spaces demonstrated in the electron micrographs of this preparation.

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EXPLANATION OF PLATE

Part of a transverse section of one of the connectives joining the pro- and mesothoracic ganglia in *Carausius*. The greater part of the field is occupied by the more-or-less circular profiles of the axons (ax). These contain neurotubules and occasional mitochondria. The remainder of the field is largely occupied by glial cell layers (gc). Between these layers lie the extracellular clefts (arrows) which occasionally dilate to form large extracellular spaces (ecs), which are filled with an electron opaque deposit. The glial cell cytoplasm contains numerous microtubules (rectangle). $\times 29000$.