A STUDY OF THE
FUNCTION OF THE NEURAL FAT-BODY SHEATH IN THE STICK INSECT, *CARAUSIUS MOROSUS*

BY J. E. TREHERNE

*A.R.C. Unit of Invertebrate Chemistry and Physiology,
Department of Zoology, University of Cambridge*

INTRODUCTION

Two conflicting hypotheses have been advanced to explain the remarkable ability of the neurones of some phytophagous insect species to function in nerve cords bathed with haemolymph containing relatively low concentrations of sodium ions. The first of these attributes the maintenance of a high extraneuronal sodium level to the activity of the glial and perineurial elements (Treherne & Maddrell, 1967). According to this interpretation of the available experimental evidence, largely derived from research on *Carausius morosus*, the necessary concentration gradient maintained between the fluid immediately surrounding the central neurones and the haemolymph results from the active extrusion of sodium ions by the glial membranes into the restricted extraneuronal channels. An alternative hypothesis which has been advanced more recently (Weidler & Diecke, 1969, 1970) is that the extraneuronal sodium regulation in *C. morosus* is achieved by the active transport of sodium ions from the haemolymph by the extraneural fat-body sheath which envelopes the central nervous system of this insect (Maddrell & Treherne, 1966).

This communication represents an attempt to test the validity of the latter hypothesis by the comparison of the effects of various concentrations of sodium ions on axonal function in intact abdominal connectives and in those subjected to varying degrees of experimental surgery.

METHODS

The modified 'sucrose-gap' technique (Stämpfli, 1954) was employed to record compound action potentials in isolated connectives from between pro- and mesothoracic ganglia. The nerve chamber used in these experiments consisted in essence of five parallel compartments isolated from one another by petroleum jelly seals as used in previous investigations from this laboratory (Treherne *et al.* 1970; Pichon & Treherne, 1970). The central compartment contained flowing experimental solution. As in a previous investigation (Pichon & Treherne, 1970) the adjacent left-hand compartment contained flowing isotonic mannitol solution, the latter substance being preferred to sucrose because it is less liable to ionic contamination. The central compartment, containing the experimental solution, was connected to the indifferent electrode by a flexible saline-filled agar bridge constructed from Nylon tubing. The left-hand compartment contained normal *Carausius* saline and was connected to a high-impedance amplifier by a second flexible saline-agar bridge.

The axons were stimulated through silver-wire electrodes in two adjacent right hand compartments by rectangular pulses (0·1 msec duration) at low impedance via an R.F. isolating unit. This apparatus was used in conjunction with a Tektronix 561 oscilloscope, a Smith Servoscribe potentiometric recorder (for continuous d.c. recordings) and a PC-21A Nihon Kohden continuous recording camera.

As in a previous investigation (Treherne *et al.* 1970), perfusion of the nerve chamber was achieved using a gravity-feed system at a rate of 5–10 ml/min from a series of elevated reservoirs. Rapid solution change with minimal mixing was achieved using a multiway non-return valve (Sattelle, 1971).

Three basic experimental preparations were used in this investigation: (i) connectives with both neural fat-body sheath and underlying nerve sheath (neural lamella and perineurium) intact, (ii) connectives with the fat-body sheath removed and nerve sheath intact, and (iii) connectives which had both fat-body sheath and nerve sheath removed. The operations for the removal of the above structures were performed on isolated nerve cords in the high-sodium saline (180 mM/l Na^+) using electrolytically sharpened tungsten needles and fine dissecting scissors. The removal of the nerve sheath was confined to a small area involving not more than 1 mm of connective.

In some experiments preparations were used in which small de-sheathed areas of connective were contained within intact lengths of neural fat-body sheath. Two slightly different operative techniques were used to achieve this. In some experiments (procedure A) the neural fat-body sheath was cut with a circumferential incision close to the mesothoracic ganglion. The tubular sleeve of fat-body sheath was then gently pushed back and a small area of the exposed nerve sheath was removed. The fat-body sleeve was then carefully drawn over the de-sheathed area. The connective was mounted in the nerve chamber with the portion containing the de-sheathed portion positioned in the central compartment containing the experimental solutions. The circumferentially cut end of the fat-body sheath was buried in the petroleum-jelly seal between the adjacent compartments. An alternative technique (procedure B) involved the making of two circumferential incisions in the neural fat-body sheath – one adjacent to the mesothoracic ganglion and the other approximately midway between the two ganglia. The posterior length of fat body was then removed and a small area of the exposed connective was de-sheathed close to the cut end of the remaining fat-body sleeve. The whole of the anterior length of neural fat-body sheath was then gently drawn posteriorly so as to cover the de-sheathed area. As previously, the connective was mounted in the nerve chamber so that the de-sheathed area underlying the repositioned fat-body sheath was placed in the central compartment containing the experimental solutions. Both of the cut ends of the tubular fat-body sleeve were buried in the petroleum jelly seals between the adjacent compartments.

The normal physiological solution employed in these experiments was that devised by Wood (1957) and had the following composition (mM/l): Na, 15; K, 18; Mg, 50; Ca, 7·5; H_2PO_4, 6; HPO_4, 4·5; Cl; together with 69·9 g/l of sucrose. Variations in the sodium concentration of the solution were accommodated by suitable alteration in the sucrose content, with the exception of sodium-free solutions when tris chloride was substituted for NaCl.

The material for structural studies was fixed at room temperature in Karnovsky's (1965) formaldehyde–glutaraldehyde solution, post-osmicated, carried through an

ascending series of ethanols to propylene oxide and embedded in Araldite. Sections were cut on an LKB Ultratome III. Thick sections (1–2 μm) for examination by the light microscope were stained with toluidine blue–borax solution. Ultra-thin sections for fine structural observations were stained with uranyl acetate and lead citrate and examined in a Philips E.M. 300.

RESULTS

The results obtained using the modified sucrose-gap technique confirm those of previous investigations on *Carausius* axons using intracellular (Treherne & Maddrell, 1967) and extracellular recording methods (Weidler & Diecke, 1969). Most importantly it has been confirmed that axonal function appears to depend upon a relatively high concentration of sodium ions in the extra-axonal fluid. This is demonstrated by the
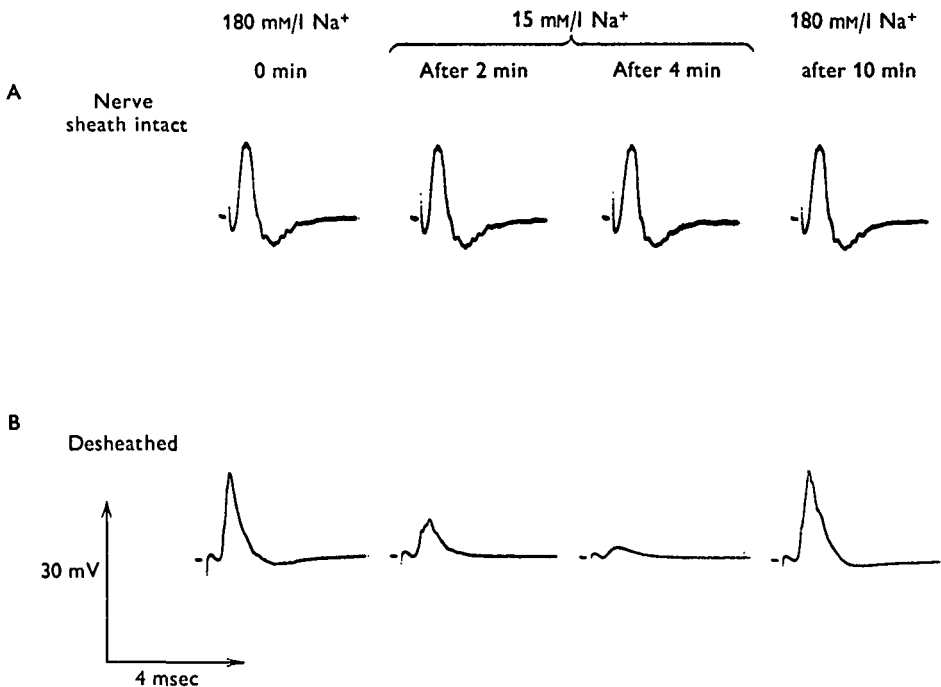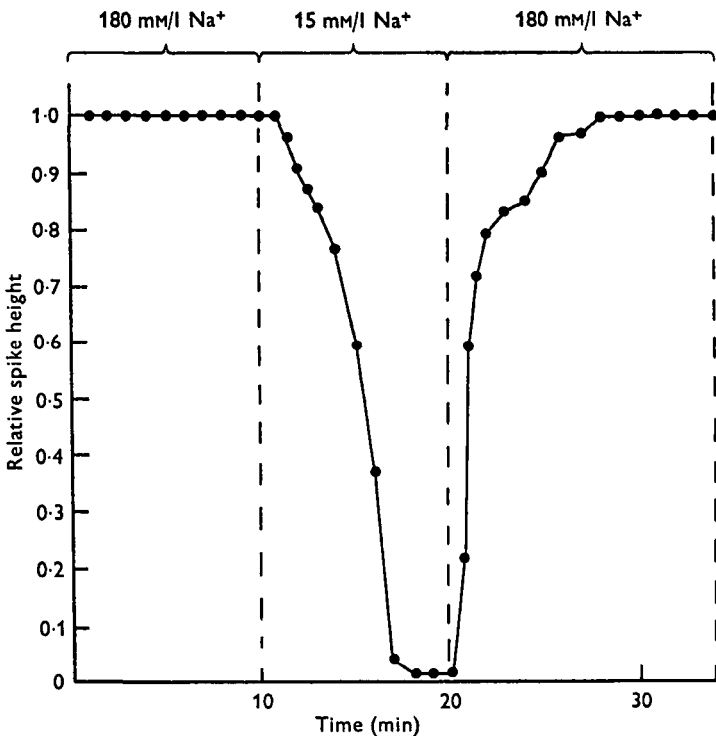

Text-fig. 1. Action potentials, recorded using the modified sucrose-gap technique, from isolated connectives of *Carausius*, showing the effects of physiological salines containing an elevated sodium concentration (180 mM/l) and a saline equivalent to the normal haemolymph level (15 mM/l). (A) A preparation in which the neural fat-body sheath was removed, the underlying nerve sheath being intact. (B) A preparation in which both the neural fat-body sheath and the underlying connective tissue and cellular nerve sheath were removed.

decline in the amplitude of action potentials recorded in connectives, from which the neural fat-body sheath and the nerve sheath had been removed, when bathed in a solution of sodium concentration equivalent to that of the haemolymph (i.e. 15 mM/l). This phenomenon is illustrated in Text-figs. 1 and 2. These diagrams also illustrate the rapid increase in the amplitude of the action potentials recorded in the presence of the high-sodium solution (180 mM/l) in these preparations.

The above results contrast with those obtained with connectives in which the nerve
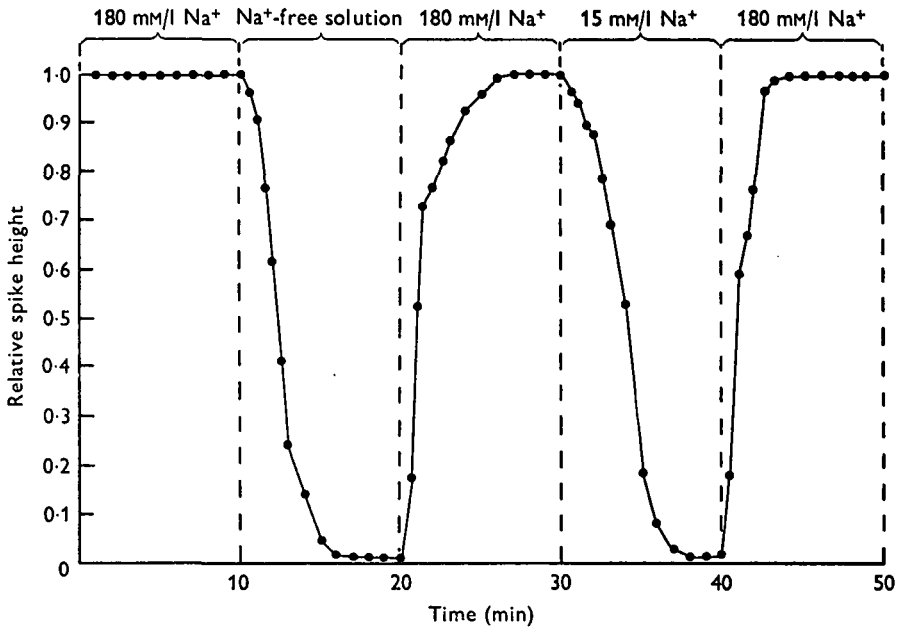
Text-fig. 2. The effects of a solution of normal haemolymph concentration of sodium ions (15 mM/l) of a solution containing an elevated concentration of this cation (180 mM/l) and of sodium-free solution on the amplitude of action potentials recorded in a de-sheathed connective in the absence of the neural fat-body sheath.

Text-fig. 3. The effect of normal (15 mM/l) and elevated (180 mM/l) sodium concentrations on action potentials recorded in an isolated connective in which a small de-sheathed area was contained within an intact length of neural fat-body sheath. This preparation was obtained using procedure A (referred to in the Methods section).

sheath was intact. With this preparation, in which only the neural fat-body sheath was removed, it will be seen that there was no appreciable decline in the amplitude of the action potentials when bathed in physiological solution containing 15·0 mM/l Na⁺ (Text-fig. 1).

Text-figs. 3 and 4 illustrate the results obtained in preparations in which the fat-body sheath was repositioned over small de-sheathed areas of connectives and described under Methods. In the presence of normal physiological saline (15 mM/l Na⁺) it was found that there was a rapid decline in the amplitude of the action potentials, with a return to full amplitude when bathed with 180 mM/l Na⁺. Essentially similar results were obtained with both methods of handling the fat-body sheath during the de-sheathing procedure.
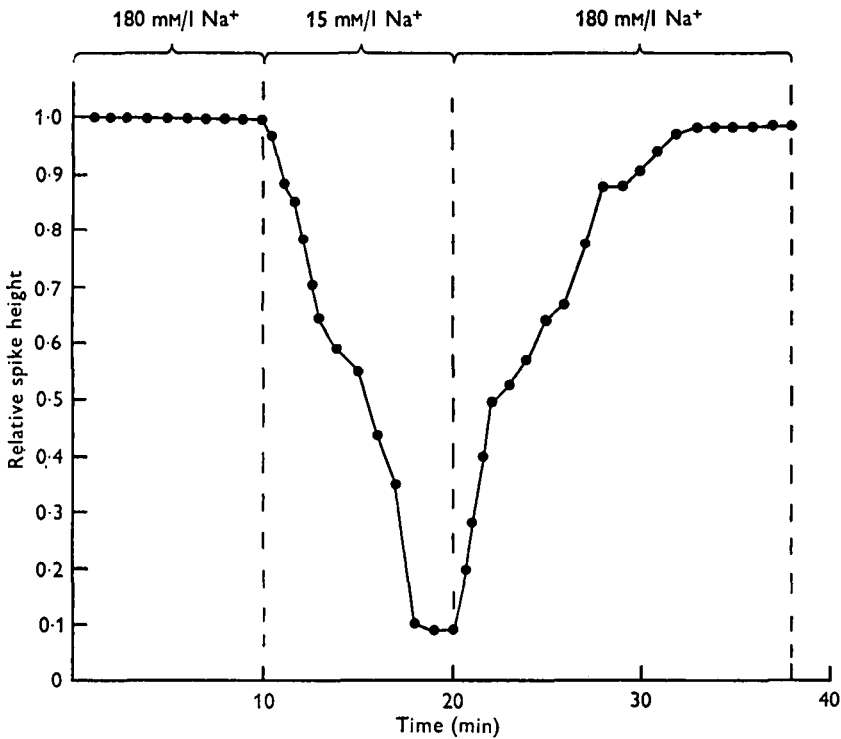
Text-fig. 4. The effects of normal (15 mM/l) and elevated (180 mM/l) sodium concentration on the amplitude of action potentials recorded in a locally de-sheathed connective contained within an intact length of neural fat-body sheath. Preparation made using procedure B (referred to in the Methods section).

Electron and light micrographs of these experimental preparations showed no significant differences in structural appearance from unoperated control nerve cords. In both cases the basement membranes were intact (Pl. 1, Figs. 1, 2). The ultra-structure of the plasma membranes of the fat-body cells and the cell constituents also appeared to be generally unaltered in the experimental preparations.

The relatively large space between the fat-body sheath and the neural lamella shown

in the light micrographs may be artifactual. In a previous investigation it has been shown that in peroxidase-injected insects the regions in which the fat-body sheath was applied to the neural lamella were completely filled with reaction product (Lane & Treherne, 1971). Where larger extraneural spaces were observed peroxidase was not present, except as a thin layer associated with the inner surface of the fat-body connective tissue and the outer edge of the neural lamella, suggesting that the large extraneural spaces may be shrinkage artifacts.

DISCUSSION

In the above experiments the ionic composition of the fluid in the compartment delimited by the neural fat-body sheath was measured using the electrical responses of axons contained in small de-sheathed areas of the underlying connectives. It was shown that the presence of the overlying neural fat-body sheath had no significant effect upon the axonal responses recorded in these preparations when bathed in low-sodium media. The decline in the amplitude of the action potentials recorded both in sodium-free conditions and at the haemolymph concentrations of 15·0 mM/l occurred as rapidly in the presence of the fat-body sheath as in completely isolated de-sheathed preparations.

These results cannot reasonably be attributed to structural damage to the neural fat-body sheath during the operative procedures, for both light-microscope and ultrastructural examination failed to reveal any significant differences between these and normal control preparations.

The present results thus indicate, first, that the neural fat-body sheath does not appear to function as an appreciable barrier to the movement of sodium ions, and secondly, that it is not apparently involved in any active regulation of the sodium concentration within the underlying fluid compartment.

Both of these conclusions accord with the results of a recent investigation which showed that particles as large as those of indian ink and peroxidase (M.W. 40,000) could penetrate the fat-body sheath (Lane & Treherne, 1971). In the case of the latter substance this penetration clearly occurred via the intercellular channels of the fat-body sheath. The rapid changes in sodium concentration which can be inferred from the present results to occur in the fluid bathing the inner surface of the fat-body sheath are, therefore, in essential agreement with the very leaky nature of this cellular layer demonstrated in the earlier investigation.

The apparent lack of any significant regulation of the sodium concentration in the extraneural fluid compartment contained by the fat-body sheath also accords with the conclusion advanced in the previous investigation (Lane & Treherne, 1971). These observations do, however, controvert the suggestion by Weidler & Diecke (1969) that the extra-axonal sodium regulation is achieved by the active transport of sodium ions across the fat-body sheath of Carausius.

The hypothesis of Weidler & Diecke (1969) has also been shown to require an improbably high rate of sodium extrusion at the inner surface of the fat-body sheath to maintain their postulated steep concentration gradient of sodium across such a leaky cellular layer (Lane & Treherne, 1970). This hypothesis does in any case seem to represent an unnecessary complication, for Weidler & Diecke, 1969, have overlooked

the fact that the fat-body sheath is absent from the peripheral nervous system of this insect (Lane & Treherne, 1971).

In view of the above considerations it would seem most reasonable to suppose that the neural fat-body sheath in *Carausius* subserves a similar function to that of fat deposits in other parts of the insect body; that is, a primary metabolic role. This supposition would also accord with the views advanced as to the role of local fat-body deposits associated with the cockroach nerve cord (Smith & Treherne, 1963).

The absence of any significant sodium regulation by the neural fat-body sheath implies that the maintenance of the relatively high extraneural concentration of this cation must be mediated within the central nervous system. Whatever the nature of the regulating system it seems clear that its effectiveness would be enhanced by a restriction of ion leakage from the extracellular fluid of the central nervous system. Such a restrictive function has, in fact, been attributed to intercellular occlusions at the inner margin of the perineurium in *Periplaneta americana* and *Carausius morosus* (Maddrell & Treherne, 1967). The occlusions appear in the former species to prevent the inward penetration of peroxidase (Lane & Treherne, 1970) and, by restricting access of inorganic ions to the inwardly facing surface of the perineurium, have been postulated to give rise to substantial extra-neuronal potentials in elevated concentrations of cations such as potassium, rubidium and caesium (Treherne *et al.* 1970; Pichon & Treherne, 1970; Pichon, Moreton & Treherne, 1971). It would seem reasonable to suppose that the sustained axonal function demonstrated in intact connectives in sodium-deficient media, both in this and in earlier investigations (Treherne, 1967; Treherne & Maddrell, 1967; Weidler & Diecke, 1969) results from such a peripheral restriction of sodium loss from the bathing medium.

According to the interpretation of Treherne & Maddrell (1967) and Treherne & Moreton (1970) the inability of the axons of *Carausius* to function at a sodium concentration equivalent to that of the haemolymph in de-sheathed connectives results not only from the removal of a peripheral perineurial diffusion barrier, but also from the disruption of glial function. It has been shown, in *Periplaneta* for example, that the de-sheathing procedure involves the removal of the neural lamella together with substantial damage to the underlying perineurium (Lane & Treherne, 1969, 1970). The latter investigation has also shown that this perineurial damage renders the underlying glial system accessible to molecules as large as those of peroxidase (M.W. 40000). Additional electro-physiological data (Treherne *et al.* 1970) suggests that this effect might result from the existence of low-resistance pathways between glial cells such as has been postulated in the central nervous system of the leech (Kuffler & Potter, 1964). The presence of such glial low-resistance pathways could conceivably be involved in the regulation of the extra-axonal sodium concentration by facilitating the intracellular movement of sodium ions from the perineurial cells to glial membranes in the region of the axon surface.

At the present time, in fact, it still seems reasonable to interpret the existing data in terms of a glial-mediated extra-axonal sodium-regulating system, as originally proposed by Treherne (1967) and Treherne & Maddrell (1967).

SUMMARY

1. The effects of variation in the sodium concentration of the bathing media on axonal function has been measured in de-sheathed connectives in the presence of the overlying neural fat-body sheath.

2. The response to solutions of the same sodium concentration as the haemolymph (15 mM/l) was found to be essentially similar to that recorded in de-sheathed connectives in the absence of the fat-body sheath, there being a rapid decline in amplitude of the recorded action potentials in both preparations.

3. On the basis of these observations it is concluded that the neural fat-body sheath is unlikely to be involved in the regulation of the extra-neuronal sodium level.

REFERENCES

KARNOVSKY, M. J. (1965). Formaldehyde–glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* 27, 137a.

KUPPLER, S. W. & POTTER, D. D. (1964). Glia in the leech central nervous system, physiological properties and neuron-glial relationships. *J. Neurophysiol.* 27, 290–320.

LANE, N. J. & TREHERNE, J. E. (1969). Peroxidase uptake by glial cells in desheathed ganglia of the cockroach. *Nature, Lond.* 223, 861–2.

LANE, N. J. & TREHERNE, J. E. (1970). Uptake of peroxidase by the cockroach central nervous system. *Tissue and Cell* 2, 413–25.

LANE, N. J. & TREHERNE, J. E. (1971). The distribution of the neural fat body sheath and the accessibility of the extraneural space in the stick insect, *Carausius morosus. Tissue and Cell* (in the Press).

MADDRELL, S. H. P. & TREHERNE, J. E. (1966). A neural fat-body sheath in a phytophagous insect (*Carausius morosus*). *Nature, Lond.* 211, 215–16.

MADDRELL, S. H. P. & TREHERNE, J. E. (1967). The ultrastructure of the perineurium in two insect species, *Carausius morosus* and *Periplaneta americana. J. Cell. Sci.* 2, 119–28.

PICHON, Y. & TREHERNE, J. E. (1970). Extraneural potentials and potassium depolarization in cockroach giant axons. *J. exp. Biol.* 53, 485–93.

PICHON, Y., MORETON, R. B. & TREHERNE, J. E. (1971). A quantitative study of the ionic basis of extra-neuronal potential change in the central nervous system of the cockroach, *Periplaneta americana. J. exp. Biol.* (in the Press).

SATTELLE, D. B. (1971). Neural function in freshwater gastropods. Ph.D. thesis, University of Cambridge.

SMITH, D. S. & TREHERNE, J. E. (1963). Functional aspects of the organization of the insect nervous system. In *Advances in Insect Physiology*, vol. 1 (ed. J. W. L. Beament, J. E. Treherne and V. B. Wigglesworth), pp. 401–84. London and New York: Academic Press.

STÄMPFLI, R. (1954). A new method for measuring membrane potential with external electrodes. *Experientia* 10, 508.

TREHERNE, J. E. (1967). Axonal function and ionic regulation in insect central nervous tissues. In *Insects and Physiology*, ed. J. W. L. Beament and J. E. Treherne. Edinburgh and London: Oliver and Boyd.

TREHERNE, J. E., LANE, N. J., MORETON, R. B. & PICHON, Y. (1970). A quantitative study of potassium movements in the central nervous system of *Periplaneta americana. J. exp. Biol.* 53, 109–36.

TREHERNE, J. E. & MADDRELL, S. H. P. (1967). Axonal function and ionic regulation in the central nervous system of a phytophagous insect (*Carausius morosus*). *J. exp. Biol.* 47, 235–47.

TREHERNE, J. E. & MORETON, R. B. (1970). The environment and function of invertebrate nerve cells. *Int. Rev. Cytol.* 28, 45–88.

WEIDLER, D. J. & DIECKE, F. P. J. (1969). The role of cations in conduction in the central nervous system of the herbivorous insect, *Carausius morosus. Z. vergl. Physiol.* 64, 372–99.

WEIDLER, D. J. & DIECKE, F. P J. (1970). The regulation of sodium ions in the central nervous system of the herbivorous insect *Carausius morosus. Z. vergl. Physiol.* 67, 160–78.

WOOD, D. W. (1957). The effects of ions upon neuromuscular transmission in a herbivorous insect. *J. Physiol., Lond.* 128, 119–39.
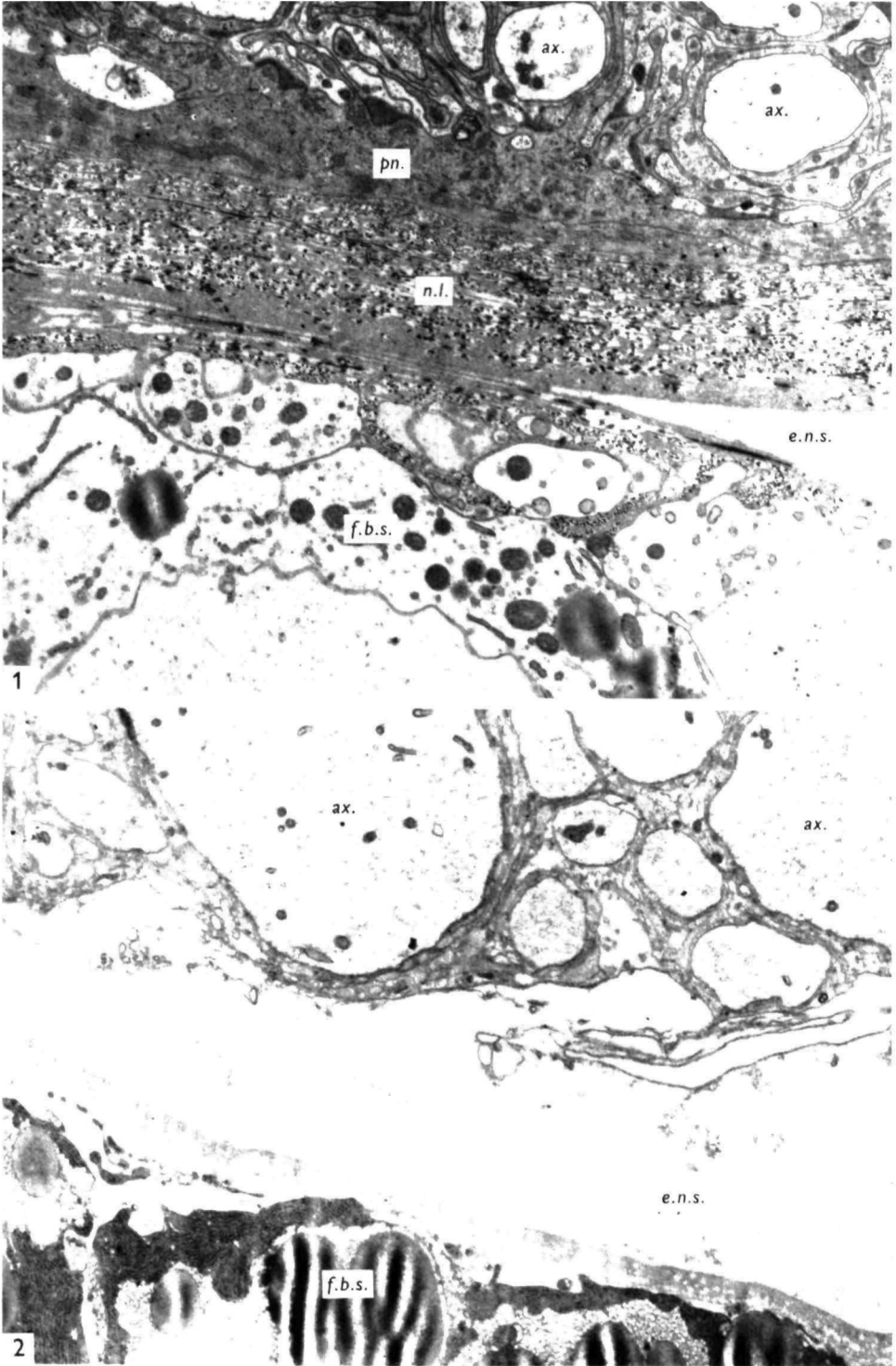
EXPLANATION OF PLATE

Electron micrographs of the outer margin of connectives and associated fat body sheaths.

Fig. 1. Unoperated preparation showing the neural fat-body sheath (*f.b.s.*) and the extra-neural space (*e.n.s.*) delimited between it and the underlying neural-lamella (*n.l.*). The perineurium (*p.n.*) is shown beneath the neural lamella. Axons (*ax.*) are shown surrounded by glial elements. × 9000.

Fig. 2. An experimental preparation in which the neural fat-body sheath has been repositioned over a de-sheathed area of the underlying connective. The de-sheathing procedure has involved the removal of substantial portions of the perineurium with the neural lamella. × 7400.