The purified carbonic anhydrase B was immobilized in polyacrylamide gel by entrapment process[12]. The enzyme was mixed with varying amounts of monomer, containing 5% cross linking material, in 0.1 M Tris-sulphate buffer, pH 7.5. Immediately after addition of the catalyst mixture and enzyme protector (N,N,N′,N′-tetramethylethylenediamine, 0.005 ml/ml of gel; ammonium persulphate, 3 mg/ml of gel; cysteine and histidine 1 mg each/ml of gel, which act as the protector of the enzyme during polymerization; dissolved in 2.0 ml of the buffer) the solution was poured into the reaction vessel and polymerization was carried out under fluorescent light for 30 min at 0–5 °C. After polymerization the polymer was crushed, washed with the same buffer and dried in the cold. Protein content of the enzyme beads was estimated according to Spackman et al.[13]. The assay of the enzyme was performed by determining its esterase activity using p-nitrophenylacetate as the substrate[14]. Thermostability of the immobilized enzyme was tested as follows: Samples of soluble and immobilized enzymes were preincubated at different temperatures from 5 °C to 80 °C for 90 min prior to incubation with p-nitrophenylacetate at 25 °C for 10 min.

Results and discussions. Carbonic anhydrase B may readily be purified by using sulphanilamide-linked polyacrylamide gel. The purification of the enzyme was noted by comparing the specific activity of the enzyme before and after chromatographic purification. The specific activity was increased from 1.2 U/mg protein to 18 U/mg protein. The table illustrates the optimum concentration of the polymer (20%) for the entrapment process. At this concentration of the gel, the 'enzyme beads' show optimum activity of the enzyme. Part of this activity may be lost in the process of polymerization. The recovery of some of the activity was found after addition of cysteine and histidine at the time of polymerization. It is known from the 3 dimensional structure of the enzyme that a number of histidine molecules, in the surroundings of the zinc ion of the active centre, play an important role in its activity[15]. It may so happen that the externally added histidine protects the free radical damage of the enzyme-histidine in the process of polymerization. The values of Km (5.9 mM for soluble and 5.6 mM for immobilized enzyme) and of pH optimum (8.0 for soluble and 7.7 for immobilized enzyme) were comparable. These suggest that the affinity to the substrate may not be affected by the entrapment process. It was found that 2×10^{-5} M sulphanilamide, a potent inhibitor of the enzyme, had no inhibitory effect on the immobilized enzyme, but 50% of the activity of the soluble enzyme was destroyed at the same concentration of the inhibitor. At the concentration of 5×10^{-4} M sulphanilamide, the activity of the soluble enzyme was completely destroyed, but the immobilized enzyme still showed 40% of the activity. The figure illustrates that about 50% of the activity of the immobilized enzyme was maintained even after preincubation at 80 °C for 90 min, while under the same condition no activity of the soluble enzyme was found. Thus the entrapment process in polyacrylamide gel may protect the active site of the carbonic anhydrase B from the effects of temperature and sulphanilamide action.

Activity and extent of entrapment of carbonic anhydrase B in polyacrylamide gel (average of 2 separate preparations)

Polymer (dry wt/100 ml of wet gel)	Added enzyme (mg/g of gel)	Protein content (mg/g of dry gel)	Bound enzyme (%)	Activity (U/g of gel)	Activity (U/mg of bound protein)
15	6.65	4.25	64	35.3	8.3
20	6.25	4.05	77	43.8	10.8
25	4.27	2.95	69	24.1	8.2

1 unit (U) is expressed as μmole of p-nitrophenol liberated per min at 25 °C.

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Studies on peripheral neurons and neurohaemal tissue in the thorax of the stick insect (*Carausius morosus*)[1]

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Summary. The distribution of peripheral neurosecretory and non-neurosecretory neurons, and transverse nerve neurohaemal swellings, is described in the thorax of *Carausius morosus*. The electrical activity of the isolated transverse nerves has been recorded.

The presence of neurosecretory and non-neurosecretory neurons lying on peripheral nerves in insects has now been described in several orders[2–7]. The most extensive study of this system has been made in the abdominal segments of the stick insect, *Carausius morosus*[2,3]. In this insect the 2 types of cells have a very different morphology and ultrastructure, and consequently it is easy to distinguish between them using methylene blue stain[3]. In this paper we describe the presence of both neurosecretory and non-neurosecretory neurons lying on major nerves in the thorax of the stick insect, as well as neurohaemal swellings which occur on the lateral branches of the median nervous system. Finlayson and Osborne[8] have shown that spontaneously generated action potentials can be recorded from the isolated neurohaemal tissue on the abdominal transverse nerves, and they have suggested

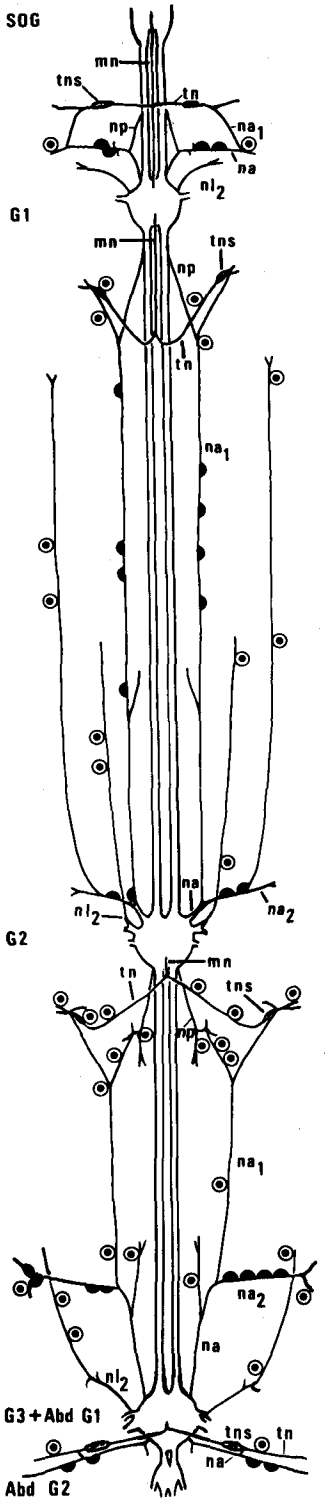

Fig. 1. Diagram of the distribution of neurosecretory neurons (●), non-neurosecretory neurons (⊙), and transverse nerve neurohaemal swellings (tns) lying on peripheral nerves in the thorax of the stick insect, Carausius morosus. na, na_1, na_2, np, Nerves labelled according to Marquhardt's terminology[12]; G1, prothoracic ganglion; G2, mesothoracic ganglion; G3 + Abd G1, metathoracic ganglion fused with abdominal ganglion 1; Abd G2, abdominal ganglion 2; mn, median nerve; SOG, sub-oesophageal ganglion; tn, transverse nerve. The left and right sides show examples of the variation in absolute position.

that the electrical activity originates in the neurosecretory endings. Similarly, electrical activity has been recorded from the neurosecretory and non-neurosecretory neurons which lie in the abdomen[9,10].

Materials and methods. Adult and larval specimens of the stick insect *Carausius morosus* were prepared for methylene blue staining and electron microscopy as previously described[2]. Neurophysiological recordings were obtained from semi-isolated or completely isolated preparations of the mesothoracic transverse nerves in saline using glass suction electrodes, the signals being amplified and displayed conventionally.

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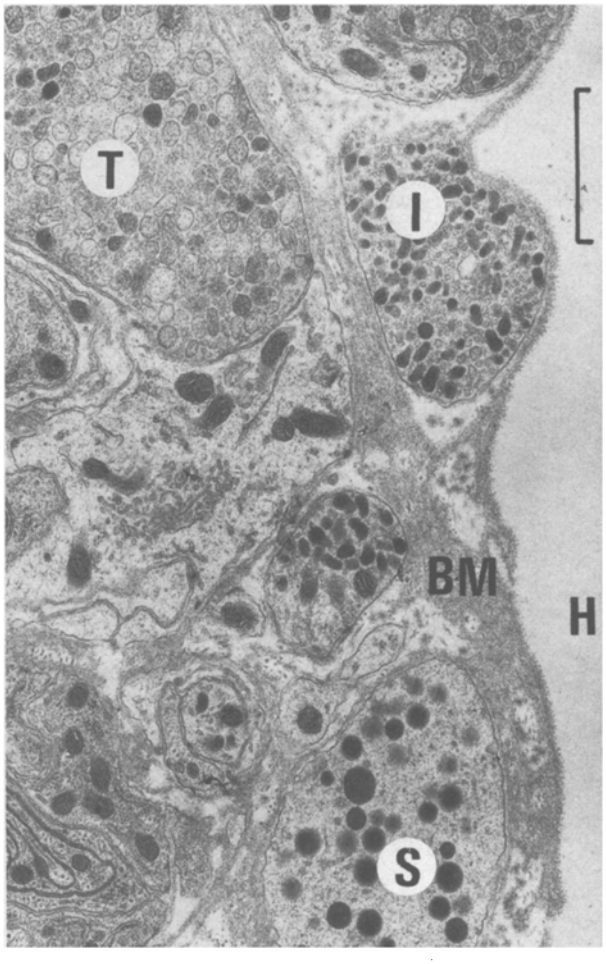

Fig. 2. Transverse section through the neurohaemal organ on the mesothoracic transverse nerve of *Carausius*. 3 types of neurosecretory axons are present containing either tubulous (T), irregular (I) or spherical (S) granules. The neurosecretory axons are separated from the haemolymph space (H) by only a thin layer of basement membrane material (BM). Scale bar: 2 µm.

Results and discussion. The distribution of peripheral neurosecretory and non-neurosecretory cells in the thorax of the stick insect is shown in figure 1. The non-neurosecretory multipolar neurons lie superficially on the peripheral nerves in fairly well-defined positions, although their exact positions is subject to individual variation (figure 1). In the meso- and meta-thoracic segments, up to 6 and 11 non-neurosecretory neurons respectively were found in each half segment, but in the much shorter prothoracic segment only 2 non-neurosecretory neurons were found. In particular we have noted the presence of non-neurosecretory neurons lying on the transverse nerves in the meso- and meta-thoracic segments. Similar neurons have been found on the transverse nerves in abdominal segments 3–8[3].

The neurosecretory multipolar neurons lie as a group in each half segment (figure 1). They lie on the link nerve in the mesothoracic segment, but in the pro- and metathoracic segments they have moved onto nerves na and na_2, as indeed they have done also in the very reduced 1st abdominal segment (figure 1). Processes pass from all of the neurosecretory neurons in numerous directions from the cell bodies along a variety of segmental nerves, including the transverse nerves.

Methylene blue preparations of the thorax also reveal the existence of typical, paired, segmentally arranged, neurohaemal swellings on the lateral branches of the median nerve (transverse nerve). These swellings lie well out in the periphery at the junction with the link nerve (figure 1).

Fig. 3. 'Spontaneously' generated action potentials recorded from 2 preparations of isolated mesothoracic transverse nerve of *Carausius* in the immediate vicinity of the neurohaemal swelling. Time mark: 0.5 sec.

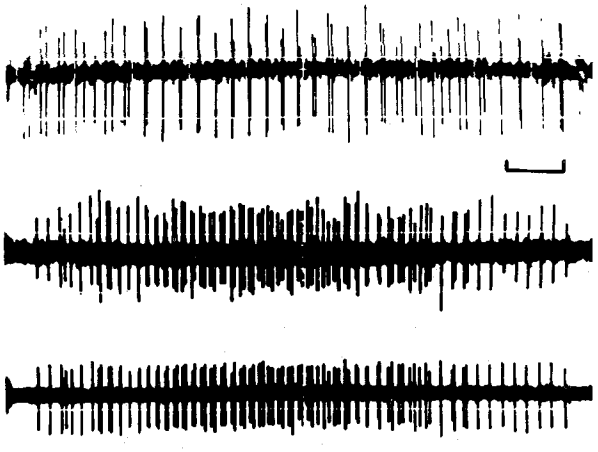

Fig. 4. 'Spontaneous' activity recorded from three isolated preparations of the mesothoracic transverse nerve of *Carausius* close to where, in the intact animal, the transverse nerve joins the median nerve. Note the apparent 'beating' rhythm caused by the 2 neurons firing with different frequencies. Time mark: 1 sec.

Transverse nerve neurohaemal swellings (perisympathetic organs) have previously been reported absent from the thorax of the stick insect[11] and so confirmation of their neurosecretory nature was obtained with the electron microscope. Figure 2 shows part of a section through the swelling on the transverse nerve of the mesothoracic segment, and illustrates typical neurohaemal tissue. 3 types of neurosecretory fibres may be recognized by their neurosecretory granules, and these are the same 3 types as are found in the abdominal transverse nerves[2,3]. They are: spherical, irregular and tubulous granules. The spherical granules are chracteristic of the link nerve neurosecretory neurons[3] and confirm the earlier observation that these cells have processes passing into the transverse nerve. The irregular and tubulous granules arise in axons belonging to neurosecretory cells found inside the central nervous system (CNS).

Recordings from the isolated neurohaemal swellings showed spontaneously generated action potentials of small amplitude and wide duration (5–10 msec, figure 3). These action potentials cannot be recorded from the nerve except in the immediate vicinity of the swellings because at a distance the small diameter neurosecretory axons lie within the nerve bundle rather than superficially as they do at the swelling. The results are consistent with the hypothesis of Finlayson and Osborne[8] that these action potentials are generated by the neurosecretory terminals. Recordings from the mesothoracic transverse nerve near the CNS revealed the existence of 2 large amplitude action potentials of short duration (1–2 msec) which are present when the nerve is isolated from the CNS and also when it is isolated from the periphery by its distal branches being severed near to the spiracles (figure 4). The 2 action potentials have slightly different frequencies of firing (in the range of 4–8 Hz) which results in overlap and consequently the production of a beating rhythm (figure 4). The pattern of firing may change when the nerve is isolated at the periphery, probably as a result of interference with the dendrites. We believe that the 2 action potentials arise in axons from the 2 multipolar neurons, and recordings with 2 suction electrodes revealed that their direction of propagation is towards the CNS.

The transverse nerve neurons show no consistent response to stretching of the nerve upon which they lie. They are, however, sensitive to manipulations of the spiracles and often cease to fire abruptly when the spiracles are interfered with mechanically.

We cannot state from our studies the function of the transverse nerve neurons although the possibility of their being coupled pacemakers existing outside the central nervous system cannot be ignored. We do feel it necessary to point out their existence, especially on the transverse nerves, as they may well complicate the interpretation of neurophysiological recordings from peripheral nerves. The distribution of neurosecretory and non-neurosecretory neurons, and neurohaemal swellings in the thorax is essentially very similar to that reported for the abdomen[2,3], and emphasizes the obvious importance of such a peripheral control complex to the insect.

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