[redacted]p. Biol. (1975), 63, 801–811
[redacted] 5 figures
Printed in Great Britain

AN ELECTROPHYSIOLOGICAL ANALYSIS OF EXTRA-AXONAL SODIUM AND POTASSIUM CONCENTRATIONS IN THE CENTRAL NERVOUS SYSTEM OF THE COCKROACH (*PERIPLANETA AMERICANA* L.)

BY M. V. THOMAS* AND J. E. TREHERNE
A.R.C. Unit of Invertebrate Chemistry and Physiology, Department of Zoology, University of Cambridge

(*Received* 20 *June* 1975)

SUMMARY

Simultaneous intracellular and sucrose-gap recordings showed, in contrast to previous findings, that the electrical parameters of giant axons were similar in intact and desheathed connectives bathed with the 'extracellular Ringer' of Yamasaki & Narahashi. This implies that the extra-axonal sodium concentration, *in situ*, is likely to be lower than had been previously supposed. Axonal responses showed that, despite the high blood concentration of 24·2 mM-K^+ measured by flame photometry, the effective concentration in the blood was 10–15 mM-K^+ which corresponds to the measurements made with potassium-selective electrodes. The activity of the blood potassium ions caused a marked reduction in the amplitude of the action potentials following surgical desheathing or disruption of the blood-brain barrier with hypertonic urea. It is suggested that a regulatory mechanism exists in the central nervous system which counteracts the effects of the high blood potassium level.

INTRODUCTION

Insects possess a remarkable ability to regulate the immediate ionic environment of their neurones. In herbivorous insects, such as *Carausius morosus* (Treherne & Maddrell, 1967; Weidler & Diecke, 1969) and *Manduca sexta* (Pichon, Sattelle & Lane, 1972), action potentials dependent on sodium ions are maintained in intact nerve cords, despite the very low concentrations of this cation in the blood, while in surgically desheathed preparations excitability is abolished by external ion concentrations equivalent to those of the blood. In the cockroach, in which the sodium activity of the blood is relatively high (a_{Na} = 0·088 M) (Treherne, Buchan & Bennett, 1975), extra-axonal sodium regulation is not obligatory. However, Pichon & Boistel (1967) showed that larger action potentials occurred in intact as compared with desheathed cockroach connectives in a high sodium Ringer: this was tentatively interpreted by Treherne & Moreton (1970) to indicate sodium regulation. Such a regulation has also been adduced from the relatively fast rate of movement of sodium ions to the axonal surfaces (in contrast to lithium ions which cannot gain access to the axons) and from

* Present address: Boston University Medical Center School of Medicine, 80 East Concord Street, [redacted]ston, Mass. 02118, U.S.A..

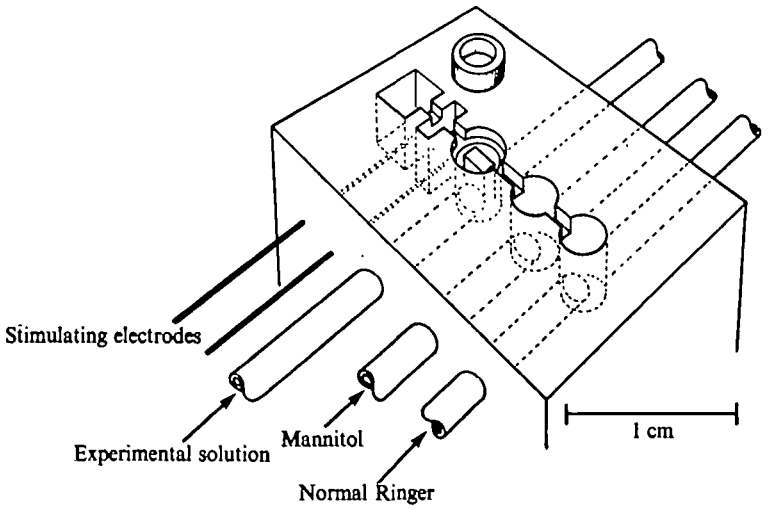

Fig. 1. Experimental chamber used for simultaneous intracellular and sucrose-gap recordings. For clarity, the vinyl ring is shown removed from its normal position in the test compartment.

the inhibition of this movement by dinitrophenol and ethacrynic acid (Schofield & Treherne, 1975).

It is conceivable that in addition to sodium regulation the extra-axonal potassium concentration may be controlled in the central nervous system of insects. This possibility, which was first recognized by Hoyle (1952), is indicated by theoretical considerations (Treherne, Lane, Moreton & Pichon, 1970) and by the effects of rapid cooling and ethacrynic acid on membrane potentials in intact and desheathed cockroach connectives (Pichon & Treherne, 1974). It is not possible, however, to predict the effects of the relatively high concentrations of blood potassium ions on the excitability of insect axons, due to insufficient characterization of axonal properties and to our ignorance of the activity of potassium ions in insect blood. This investigation was, therefore, initiated to discover the effects of blood cations on the excitability of cockroach axons, in intact and experimentally treated preparations, using sucrose-gap and intracellular microelectrode recording techniques.

METHODS

Sucrose-gap recordings were carried out using a previously described modification of the technique (Pichon & Treherne, 1970). Simultaneous intracellular and sucrose-gap recordings were made from penultimate abdominal connectives using a small perspex nerve chamber (Fig. 1). Isolated nerve cords were mounted across the five compartments, shown in Fig. 1, the adjacent compartments being isolated from each other by silicone grease seals in the grooves between the compartments. The terminal abdominal connectives were passed through the two stimulating compartments which were filled with Yamasaki & Narahashi (1959) Ringer. The penultimate connectives were supported by a small rubber block in the test compartment to facilitate microelectrode penetration. A ring cut from a length of vinyl tubing fitted into this compartment. This arrangement facilitated microelectrode penetration by holding t

connectives against the rubber block without subjecting them to undue pressure or tension. The test compartment was connected to the indifferent electrode by a KCl-Agar bridge placed in the drainage line. The extracellular sucrose-gap recordings were made between the test compartment and the right-hand one, which contained flowing Yamasaki & Narahashi Ringer, and was connected by another KCl-Agar bridge to an Ag/AgCl electrode. These two compartments were separated by flowing isotonic mannitol solution (Fig. 1).

The glass microelectrodes were filled with 3 M-KCl and had resistances of approximately 15 MΩ and tip potentials of 2–3 mV. The microelectrode and the sucrose-gap recording electrode were connected, by separate high-impedance amplifiers, to a Tektronix 502A oscilloscope and a Servoscribe RE 520.20 pen recorder. Photographs were taken using a Nihon Kohden PC2A oscilloscope camera, which was automatically controlled (Thomas, 1974). The microelectrode signal was fed to an operational amplifier differentiator circuit. The differentiated intracellular or the sucrose-gap signals were displayed on the second channel of the oscilloscope by operation of a changeover switch. The preparation was stimulated through the paired platinum wires (Fig. 1) via an RF isolating unit from a Farnell pulse-generating system.

A regulated flow of the experimental solutions and of mannitol through the appropriate compartments of the nerve chambers was achieved by a gravity-feed system from a series of elevated reservoirs. Rapid changes of solution were effected using a multiway, non-return, valve (Holder & Sattelle, 1972).

The experimental solutions used in this investigation were based on the Ringers devised by Yamasaki & Narahashi (1959) and Bennett, Buchan & Treherne (1975). The former, which was designed as an 'extracellular' Ringer, has the following composition: 214 mM-Na^+, 3·1 mM-K^+, 1·8 mM-Ca^{2+}, 216·9 mM-Cl^-, 0·2 mM-$H_2PO_4^-$, 1·8 mM-HPO_4^{2-}. The Ringer of Bennett *et al.* (1975) was designed to approximate to the ionic conditions in cockroach blood and has the following composition: 120 mM-Na^+, 25 mM-K^+, 2·0 mM-Ca^{2+}, 2·0 mM-Mg^{2+}, 19·02 mM $Tris^+$, 163·7 mM-Cl^-, 0·2 mM-$H_2PO_4^-$, 1·8 mM-HPO_4^{2-}, 2·5 mM-HCO_3^-. Reductions in the potassium concentration of the latter Ringer were accommodated by appropriate increases in the concentration of Tris. In sucrose-gap experiments, in which simultaneous intracellular measurements were made, a mannitol solution of 483 mM was used (Pichon & Treherne, 1970). In experiments using the Ringer of Bennett *et al.* (1975) a mannitol concentration of 336·2 mM was employed.

Adult male *Periplaneta americana* were used in these experiments. They were reared at a temperature of 27–29 °C, under normal daylight conditions, on a mixed diet described by Treherne, Buchan & Bennet (1975).

Blood samples were collected from restrained adult male cockroaches by pricking the front of the head, between the antennae, or at the base of a coxa and sucking the extruded blood into a glass micropipette. The sodium and potassium concentrations of the blood were measured using a Unicam SP 90 A atomic absorption spectrophotometer, 5 μl blood samples being diluted to 1 ml for this purpose. 100 μl blood samples were also collected by pooling blood from several individuals. In some sucrose-gap experiments these samples were used to test the electrical responses of urea-treated or desheathed preparations. To do this, flow was stopped in the test compartment and any remaining Ringer solution carefully removed with small pieces

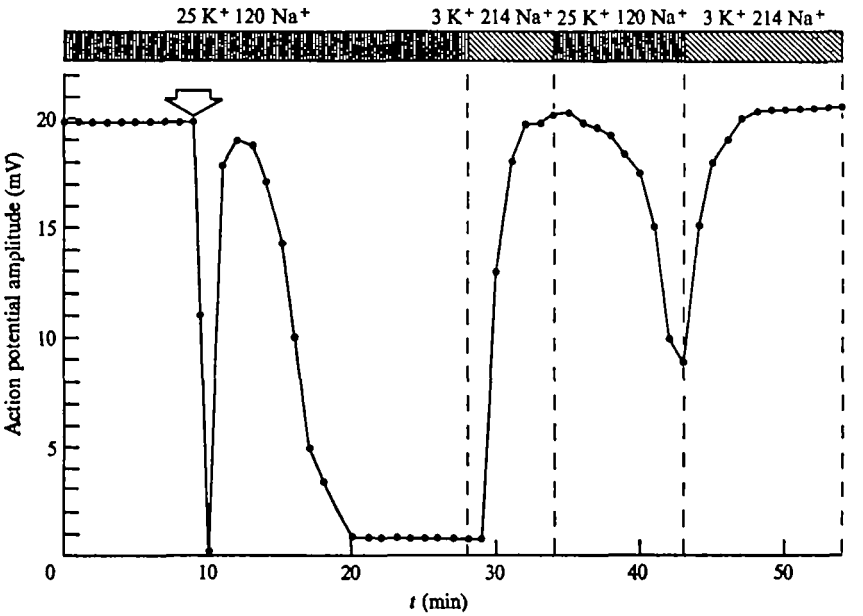

Fig. 2. The effects of disruption of the blood-brain barrier by 30 s exposure to 3·0 M urea (arrow), on the amplitude of the action potentials recorded in 'blood Ringer' (25 mM-K^+, 120 mM-Na^+) and during subsequent exposure to 'extracellular Ringer' (3 mM-K^+, 214 mM-Na^+). Sucrose-gap recording.

of Kleenex tissues. The 100 μl blood sample was then discharged into the test compartment. The blood was removed from the test compartment by a slow flow of Ringer into the compartment, any blood clots being carefully extracted with fine forceps.

RESULTS

(*a*) *Effects of 'blood' and 'extracellular' Ringer, after disruption of the blood-brain barrier*

Experiments were performed to determine the effects of disrupting the blood-brain barrier, by brief exposure to hypertonic urea solution (Treherne, Schofield & Lane, 1973), on the action potentials recorded in the 'blood Ringer' of Bennett *et al.* (1975). In this Ringer the sodium concentration (120 mM) is equivalent to the activity (a_{Na} = 0·088 M) of the blood sodium (Treherne *et al.* 1975). The relatively high potassium concentration (25 mM) in this Ringer was found to be necessary to maintain the potassium content of isolated nerve cords and was equivalent to blood concentration measured by flame photometry (Treherne *et al.* 1975). Sucrose-gap recordings showed that treatment of intact connectives with hypertonic urea caused an immediate and transient decrease in the amplitude of the action potentials (Fig. 2). The subsequent recovery was then followed by a decline and development of a conduction block. A rapid recovery was obtained with 'extracellular Ringer' (214 mM-Na^+, 3 mM-K^+), the amplitude of the action potentials approximating to those measured in the 'blood Ringer' before urea treatment. A recovery could also be obtained by reducing the potassium concentration in the 'blood Ringer' to the level used in 'extracellular Ringer' (3 mM-K^+) (Fig. 3).

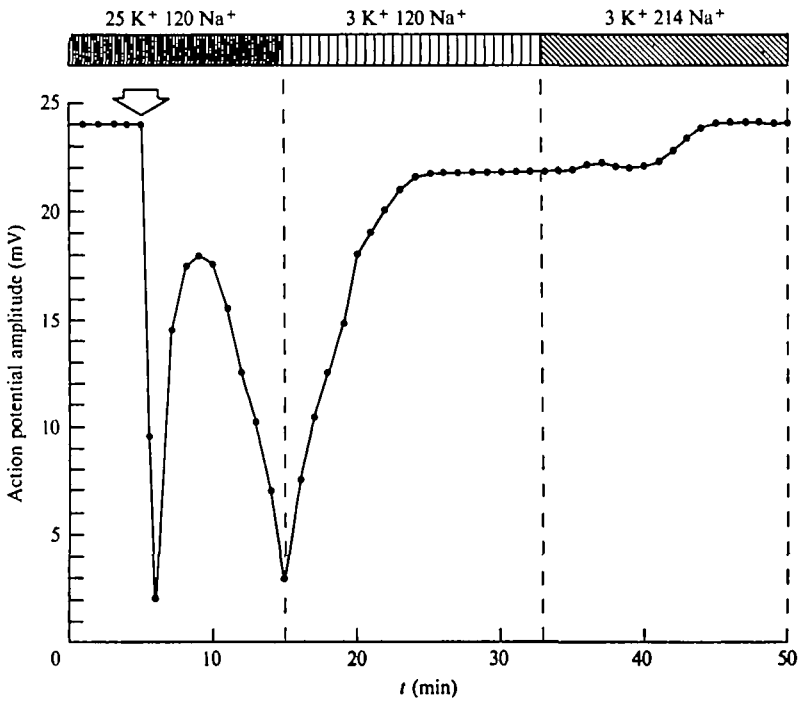

Fig. 3. The effects of exposure to hypertonic urea (arrow) on the amplitude of action potentials during exposure to 'blood Ringer' (25 mM-K^+, 120 mM-Na^+). The subsequent effects of reducing the potassium concentration (3 mM-K^+, 120 mM-Na^+) in the latter Ringer is shown, together with the effects of exposure to 'extracellular Ringer' (3 mM-K^+, 214 mM-Na^+). Sucrose-gap recording.

The above results indicate that axonal function is effectively abolished by a potassium concentration (25 mM-K^+) equivalent to that measured by flame photometry in cockroach blood (Treherne *et al.* 1975). The relatively high blood potassium concentration was confirmed by flame photometer measurements which yielded a mean value of $24{\cdot}2 \pm 0{\cdot}78$ mM-K^+.

(b) Effects of cockroach blood on desheathed and urea-treated connectives

In contrast to the effects of 'blood Ringer' exposure of urea-treated (Fig. 4) or surgically-desheathed connectives (Fig. 5) to pooled cockroach blood resulted in all cases in a decline in the amplitude of the recorded action potential, but *not* in complete conduction block. With urea-treated preparations the action potentials declined to $64{\cdot}0 \pm 3{\cdot}8\%$ ($n = 7$) and with desheathed ones to $41{\cdot}6 \pm 5{\cdot}6\%$ ($n = 5$) of the initial amplitudes. The responses of these experimentally treated preparations to blood were equivalent to those observed in the concentration range 10–15 mM-K^+, at 120 mM-Na^+ (Figs. 4 and 5), the decline in the amplitude of the action potentials being proportionally greater in the desheathed preparations.

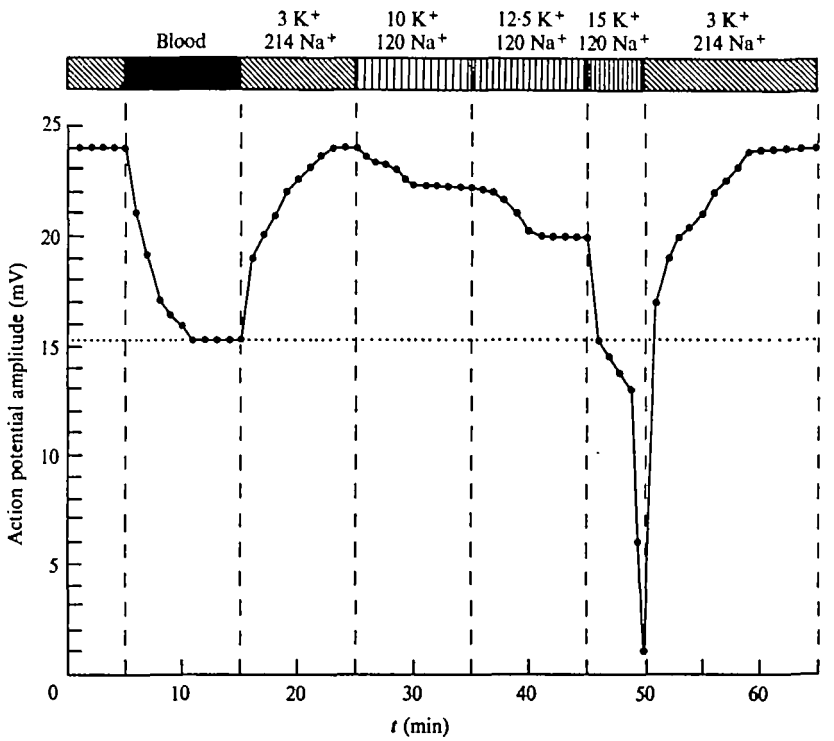

Fig. 4. The effects of exposure to freshly collected blood on the amplitude of the action potentials recorded in connectives in which the blood-brain barrier had been previously disrupted by 30 s treatment with hypertonic urea. The effects are compared with successive exposures to 'blood Ringer' (120 mM-Na^+) in which the potassium concentration was changed, from 10 to 12·5 and to 15 mM-K^+. The preparation was maintained in 'extracellular Ringer' (3 mM-K^+, 214 mM-Na^+) between exposure to the former Ringers and to blood. Sucrose-gap recording.

(*c*) *Comparison of intact, desheathed and urea-treated connectives in 'extracellular Ringer'*

The data illustrated in Figs. 1 and 2 indicated that action potentials measured in intact preparations with the 'blood Ringer' (120 mM-Na^+, 25 mM-K^+) were similar to those obtained following urea-treatment in the 'extracellular Ringer' (214 mM-Na^+: 3 mM-K^+) of Yamasaki & Narahashi (1959). These sucrose-gap observations differ from those of Pichon & Boistel (1967), who recorded action potentials of significantly smaller amplitude in intact as compared with desheathed preparations bathed in Yamasaki & Narashashi Ringer, and from those of Treherne *et al.* (1973), which showed an apparent reduction in amplitude following disruption of the blood-brain barrier with hypertonic urea. Experiments were, therefore, carried out in which simultaneous microelectrode and sucrose-gap records were obtained from 16 intact and 16 desheathed preparations bathed, as in the experiments of Pichon & Boistel (1967), with Yamasaki & Narahashi Ringer. These experiments were performed at a temperature of between 23 and 25 °C, which is within the range used by Pichon & Boistel. Measurements were taken only when all five of the parameters recorded in Table 1 had reached steady values, a process which normally took 15–30 min for desheath

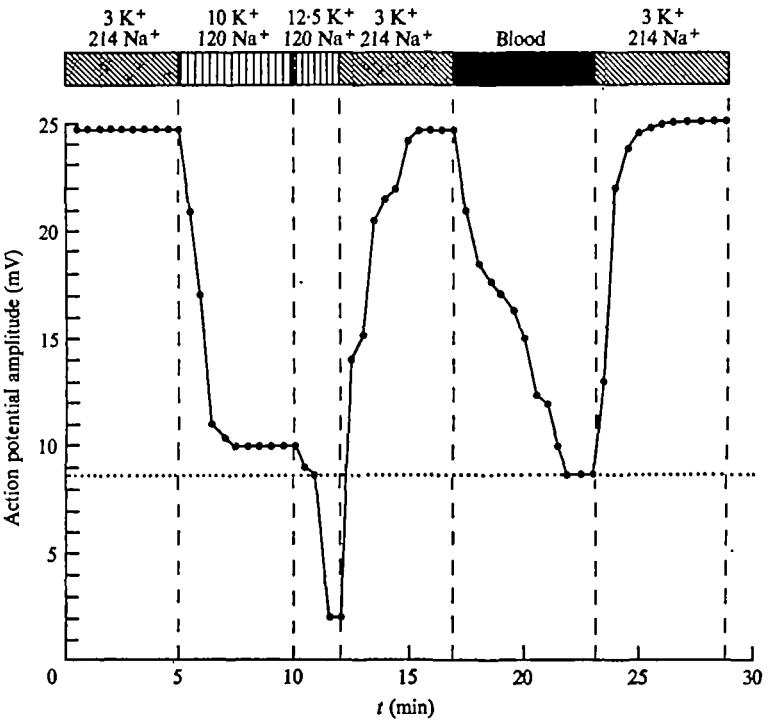

Fig. 5. Effects of exposure of desheathed connectives to 'blood Ringer' (120 mM-Na^+), at potassium concentrations of 10 and 12·5 mM, and to freshly collected blood. The preparation was kept in 'extracellular Ringer' (3 mM-K^+, 214 mM-Na^+) between exposure to the above Ringer and to blood. Sucrose-gap recording.

Table 1. *The electrical responses recorded with intracellular microelectrodes and with the sucrose-gap in intact and desheathed connectives bathed with the 'extracellular Ringer' of Yamasaki & Narahashi* (1959). *The values represent the means and standard errors. The bracketed figures are the mean values previously obtained by Pichon & Boistel* (1967)

Preparation	Resting potential (mV)	Action potential (mV)	Max. rate of rise (V/s)	Max. rate of fall (V/s)	Mannitol gap a.p. (mV)
Intact (n = 16)	71·1 ± 1·9 (58·1)	108·3 ± 1·3 (103·0)	1059 ± 37 (928·1)	464 ± 19 (321·9)	19·2 ± 1·25 —
Desheathed (n = 16)	77·8 ± 1·0 (67·4)	106·8 ± 1·2 (85·9)	984 ± 30 (804·7)	440 ± 15 (320·3)	18·6 ± 1·2 —
P	0·01	0·4	0·1	0·3	0·7

preparations when the values often rose by 10% or more. The values recorded from intact preparations showed far smaller increases.

With the exception of the resting potential none of the parameters recorded in Table 1 differ significantly between desheathed and intact preparations. The differences in the resting potentials recorded in the two classes of preparations is, however, highly significant ($P < 0·01$), the mean value in the intact preparations being 6·7 mV lower than in desheathed ones.

DISCUSSION

The above observations showed that the cation concentrations in the Ringer of Yamasaki & Narahashi (1959) (214 mM-Na^+, 3 mM-K^+, 1·8 mM-Ca^{2+}) maintained action potentials in desheathed connectives which were similar to those recorded in intact ones. The 6·7 mV difference in the mean resting potentials recorded from giant axons in intact and desheathed connectives is consistent with the previous observations of Pichon & Boistel (1967). The similarity of this potential difference to the mean value of the positive potential (the 'sheath' or 'extracellular' potential), measured with the microelectrode tip in an apparently extracellular position, suggests that the true value of the resting potential may be similar in intact and desheathed preparations bathed with Yamasaki & Narahashi Ringer. The mean action potential amplitude recorded from intact preparations (108·3 ± 1·3 mV) does not differ significantly ($P = 0·4$) from that obtained with desheathed ones (106·8 ± 1·2 mV). The much larger effects of desheathing reported by Pichon & Boistel (1967) could well have resulted from the fact that their values were recorded immediately after microelectrode impalement (Y. Pichon, personal communication). The responses recorded in this way from desheathed connectives would, thus, appear smaller than those obtained with intact ones, for the action potentials recorded from desheathed connectives show a proportionally greater increase during the 15–30 min stabilization period following impalement than do those recorded in intact connectives.

Action potentials recorded in urea-treated preparations, bathed in the Yamasaki & Narahashi Ringer, were also similar to those measured in intact conditions with the 'blood Ringer' of Bennett *et al.* (1975) (i.e. 120 mM-Na^+, 25 mM-K^+). These data, which controvert earlier observations (Pichon & Boistel, 1967; Treherne *et al.* 1973), indicate that there is no basis for postulating an elevated extra-axonal sodium concentration of the magnitude (483 mM) calculated by Treherne & Moreton (1970) from the data of Pichon & Boistel (1967). This does not necessarily imply that a sodium gradient between the extra-axonal fluid and the blood is absent in the cockroach central nervous system. The critical sensitivity of the spike-generating mechanism to potassium and calcium ions makes it difficult to predict the extra-axonal sodium concentration from the active membrane potentials of intact and desheathed or urea-treated preparations. However, the resting potential of cockroach axons is relatively insensitive to sodium (Yamasaki & Narahashi, 1959) and to divalent cations (Narahashi, 1966). The similarity of the apparent resting potential in intact preparations to that measured in desheathed ones could thus indicate that the extra-axonal potassium level *in situ* approximates to, or is less than, the concentration of this cation in the Ringer of Yamasaki & Narahashi (1959) (i.e. about 3 mM-K^+). In this case the effectiveness of the latter Ringer in mimicking the extra-axonal conditions in desheathed preparations could indicate that the extra-axonal sodium concentration might be as high as 214 mM-Na^+, as compared with an external sodium level of 120 mM-Na^+, which is equivalent to the sodium activity of cockroach blood (Treherne *et al.* 1975). Extra-axonal sodium regulation in cockroach central nervous connectives can, in any case, be inferred from the demonstration of an inwardly directed active transport of sodium ions from the external medium to the fluid bathing the axon surfaces in intact preparations (Schofield & Treherne, 1975).

The present evidence also indicates the possibility of extra-axonal cation regulation in intact nerve cords, to counteract the effects of blood potassium ions. The total potassium concentration of cockroach blood is extremely variable (cf. Asperen & Esch, 1956; Pichon, 1970), but average concentrations as high as 22·8 mM (Pichon, 1970), 24·2 mM (present investigation) and 25 mM (Treherne *et al.* 1975) have been measured by flame photometry. It is probable that a proportion of this potassium is contained within the haemocytes (Brady, 1967) although it was found necessary to use a concentration of 25 mM-K^+ to maintain the potassium content of isolated cockroach nerve cords in a 'blood Ringer' of sodium concentration (120 mM) equivalent to the measured sodium activity of the blood (Treherne *et al.* 1975). However, the present investigation has shown that this blood Ringer produced axonal conduction block following disruption of the blood-brain barrier with hypertonic urea. Freshly collected blood, on the other hand, produced a smaller effect on the amplitude of the action potentials recorded in urea-treated and desheathed preparations. This could imply that the effective potassium concentration in the plasma was less than in the 'blood Ringer', for the experiments with desheathed and urea-treated preparations, indicated that the axonal responses to blood were equivalent to those measured in the Ringer containing 10–15 mM-K^+. This comparison is complicated by the possible effects of calcium ions which at high concentrations are known to reduce the sodium-inactivation produced by potassium depolarization of cockroach axons (Narahashi, 1966). However, the effective concentration range of potassium ions estimated from the axonal responses accords with recent measurements made on cockroach blood with potassium-selective electrodes. These measurements yielded values for a_K of around 0·01 M, which correspond to an average concentration of approximately 12·5 mM-K^+ (W. A. Foster, personal communication). It may not, therefore, be necessary to assume a high activity of blood calcium, to account for the limited degree of sodium inactivation observed, on exposure of desheathed preparations to blood. The total calcium content of cockroach blood, 4·2 mM (Asperen & Esch, 1956), is, in any case, relatively low in comparison with the concentrations which have been shown to reduce Na-inactivation in depolarized axons (Frankenhaeuser & Hodgkin, 1957).

The above considerations suggest that *in situ* the effects of the relatively high external potassium level are counteracted by regulatory mechanisms within the central nervous system, for although the peripheral blood-brain barrier restricts the inward movement of potassium ions there appears, nevertheless, to be a finite leakage of this cation into the extracellular system (Pichon, Moreton & Treherne, 1971). Such a regulation could be effected in one or both of two ways. If the activity of the extra-axonal calcium was appreciably higher than in the experimental solutions (i.e. $> 1{\cdot}8$ mM) then it would tend to counteract the sodium-inactivation induced by potassium depolarization (cf. Narahashi, 1966). Alternatively, it is conceivable that *in situ* the extra-axonal potassium is maintained at a lower effective concentration than in the blood. This supposition accords with the apparent similarity of the axonal resting potentials, in intact and desheathed preparations, which, as has already been suggested, can be interpreted as indicating an extra-axonal potassium concentration of $\leqslant 3$ mM. This interpretation implies that the extra-axonal potassium concentration would be not more than one-third that of the blood. Such a potassium regulation could result from an active regulation by the glial and/or perineurial elements: a mechanism

which has been predicted from theoretical considerations (Treherne *et al.* 1970), and suggested by the effects of cooling and sodium-transport inhibitors on the membrane potentials in *intact* cockroach connectives (Pichon & Treherne, 1974). The presence of linked sodium/potassium pumps on the glial membranes can, for example, be readily conceived as being capable of reducing the potassium concentration in the narrow extracellular channels (cf. Treherne, 1974) as well as being involved in mediating net inward movements of sodium ions into them (Schofield & Treherne, 1975).

Both blood and 'blood Ringer' (10–15 mM-K^+) produced a more pronounced decline in the amplitude of the action potentials in desheathed than in urea-treated connectives. This effect could result from either of the two mechanisms discussed above, that is from some residual potassium regulation or from the retention of higher concentrations of calcium ions at the axon surfaces in the latter preparation.

We are grateful for the help and advice of Dr R. B. Moreton, Mr P. K. Schofield and Miss P. Willmer during this investigation. The illustrations were prepared by Mr J. W. Rodford.

REFERENCES

ASPEREN, K. VAN & ESCH, I. VAN (1956). The chemical composition of the haemolymph in *Periplaneta americana*. *Archs. Néerl. Zool.* II, 342–60.

BENNETT, R. R., BUCHAN, P. B. & TREHERNE, J. E. (1975). Sodium and lithium movements and axonal function in cockroach nerve cords. *J. exp. Biol.* **62**, 231–41.

BRADY, J. (1967). The relationship between blood ions and blood cell density in insects. *J. exp. Biol.* **48**, 313–26.

FRANKENHAEUSER, B. & HODGKIN, A. L. (1957). The action of calcium on the electrical properties of squid axons. *J. Physiol.* **137**, 217–44.

HOLDER, R. E. D. & SATTELLE, D. B. (1972). A multiway non-return valve for use in physiological experiments. *J. Physiol.* **226**, 2–3 *P*.

HOYLE, G. (1952). High blood potassium in insects in relation to nerve conduction. *Nature* **169**, 281–2.

NARAHASHI, T. (1966). Dependence of excitability of cockroach giant axons on external divalent cations. *Comp. Biochem. Physiol.* **19**, 759–74.

PICHON, Y. (1970). Ionic content of haemolymph in the cockroach, *Periplaneta americana*. A critical analysis. *J. exp. Biol.* **53**, 195–209.

PICHON, Y. & BOISTEL, J. (1967). Microelectrode study of the resting and action potentials of the cockroach giant axon with special reference to the role played by the nerve sheath. *J. exp. Biol.* **47**, 357–73.

PICHON, Y., MORETON, R. B. & TREHERNE, J. E. (1971). A quantitative study of the ionic basis of extraneuronal potential changes in the central nervous system of the cockroach (*Periplaneta americana* L.) *J. exp. Biol.* **54**, 757–77.

PICHON, Y., SATTELLE, D. B. & LANE, N. J. (1972). Conduction processes in the nerve of the moth, *Manduca sexta*, in relation to haemolymph ionic composition and central nervous ultrastructure. *J. exp. Biol.* **56**, 717–34.

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

PICHON, Y. & TREHERNE, J. E. (1974). The effects of sodium transport inhibitors and cooling on membrane potentials in cockroach central nervous connectives. *J. exp. Biol.* **61**, 203–18.

SCHOFIELD, P. K. & TREHERNE, J. E. (1975). Sodium transport and lithium movements across the insect blood-brain barrier. *Nature* **255**, 723–5.

THOMAS, M. V. (1974). Permeability of the insect blood-brain barrier. Ph.D. thesis, University of Cambridge.

TREHERNE, J. E. (1974). The environment and function of insect nerve cells. In *Insect Neurobiology* (ed. J. E. Treherne), pp. 187–244. Amsterdam: North-Holland.

TREHERNE, J. E., BUCHAN, P. R. & BENNETT, R. R. (1975). Sodium activity of insect blood: physiological significance and relevance to the design of physiological saline. *J. exp. Biol.* **62**, 721–32.

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**, 25–88.

TREHERNE, J. E., SCHOFIELD, P. K. & LANE, N. J. (1973). Experimental disruption of the blood-brain barrier system in an insect (*Periplaneta americana*). *J. exp. Biol.* **59**, 711–23.

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

YAMASAKI, R. & NARAHASHI, T. (1959). The effects of potassium and sodium ions on the resting and action potentials of the cockroach giant axon. *J. Insect Physiol.* **3**, 146–58.