J. Exp. Biol. (1962), 39, 631–641
With 10 text-figures
Printed in Great Britain

SOME EFFECTS OF THE IONIC COMPOSITION OF THE EXTRACELLULAR FLUID ON THE ELECTRICAL ACTIVITY OF THE COCKROACH ABDOMINAL NERVE CORD

By J. E. TREHERNE

A.R.C. Unit of Insect Physiology, Department of Zoology, University of Cambridge

(*Received* 19 *July* 1962)

INTRODUCTION

The chemical composition of the fluid surrounding the cells of the central nervous system of *Periplaneta* has been shown to differ from that of the haemolymph, due to a Donnan equilibrium which maintains an excess of inorganic cations in the extracellular fluid (Treherne, 1962 *a*, *b*). In view of the demonstrated permeability of the nerve sheath to ions and molecules (Treherne, 1961 *a*, *b*) it was suggested that the enhanced rates of potassium depolarization obtained in desheathed preparations (Hoyle, 1953; Twarog & Roeder, 1956) might be a result, in part at least, of the changed extracellular environment resulting from the disruption of the Donnan equilibrium with the haemolymph (Treherne, 1962*b*). The present investigation represents an attempt to determine the part which such ionic disturbances play in the electrical changes resulting from the removal of the nerve sheath in solutions of varying potassium concentration. In these experiments the rates of potassium depolarization of desheathed preparations irrigated with various solutions have been followed and compared with those obtained in salines in which the cation levels were elevated to correspond to their extracellular concentrations as calculated from the Donnan equilibrium. The synaptic transmission in the desheathed terminal abdominal ganglion in high concentrations of acetylcholine has also been studied in an attempt to throw some light on the factors involved in the development of conduction block under these conditions.

METHODS

In this investigation the conduction along various portions of the cockroach abdominal nerve cord was measured under a variety of experimental conditions. To test the effects of different concentrations of ions on these conduction processes small windows were cut in the integument of the ventral abdominal surface to expose the connectives between the first and second pairs and between the fifth and sixth (terminal) pairs of abdominal ganglia. Silver-wire stimulating electrodes were then placed on the anterior pair of connectives and similar recording electrodes on the posterior pair. The electrodes and connectives were covered with a layer of liquid paraffin to reduce as far as possible any drying of the preparation. Following this a third window was cut through the integument overlying the fourth abdominal ganglion and a small piece of nylon sheet was carefully slipped beneath the ganglion as described by Twarog & Roeder (1956). The exposed ganglion was then irrigated with one or other of the physiological salines used in this investigation. In some

experiments the ventral portion of the nerve sheath was removed from the ganglion by tearing with finely ground watchmakers' forceps as described by Twarog & Roeder (1956).

A second nerve preparation was used to measure transmission across the terminal abdominal ganglion. To do this a pair of stimulating electrodes of platinum wire was thrust into the right abdominal cercus, which was supported on a small platform of paraffin wax. The integument overlying the terminal ganglion and connective was then removed and the recording electrode was placed beneath the exposed connective. The terminal ganglion was continuously irrigated with the experimental saline or, in a limited number of cases, the saline was injected beneath the nerve sheath from a micro-syringe. The needle of the syringe was constructed of finely drawn glass tubing (the diameter of the tip being approximately 70μ) attached to a Scholander-type micrometer syringe.

The nerve preparations were stimulated by an uninterrupted series of rectangular pulses (0·5/sec.; 0·2 msec. duration) at low output impedance via an RF isolating unit. The recording system consisted of a differential preamplifier (Tektronix Type 122) coupled to a Tektronix Type 532 oscilloscope.

Table 1. *The concentrations of sodium, potassium and calcium ions in the solutions used in these experiments*

(The elevated cation levels, corresponding to the estimated extracellular concentrations, were calculated from data given by Treherne (1962*b*). The concentrations of the other substances present were those used in the physiological saline employed in previous investigations (Treherne, 1961*a*).)

Ions	'Basic' saline, 1A (mM./l.)	'Extracellular' saline, 1B (mM./l.)	'Basic' saline, 2A (mM./l.)	'Extracellular' saline, 2B (mM./l.)	Modified 'extracellular' saline (mM./l.) 2C	2D	'Basic' saline, 3A (mM./l.)	'Extracellular' saline, 3B (mM./l.)
Potassium	12·3	17·1	70·0	85·9	85·9	85·9	154·8	236·0
Sodium	157·0	283·6	99·3	153·0	99·3	153·0	12·3	18·0
Calcium	4·5	17·6	4·5	17·6	17·6	4·5	4·5	17·6

The composition of the salines used in the experiments on the effect of ionic concentration on nervous conduction are shown in Table 1. In these salines the progressive increase in potassium concentration was compensated for by an equivalent decrease in sodium concentration. Besides the 'basic' salines, additional 'extracellular' salines were used in which the concentrations of the cations were elevated to correspond to those postulated in the extracellular fluid of the central nervous system (Treherne, 1962 *a*, *b*).

RESULTS

When the fourth abdominal ganglion was irrigated with the normal physiological saline (saline 1A) corresponding in ionic composition to that of cockroach haemolymph, conduction along the nerve cord remained relatively constant throughout the period of the experiment (Fig. 1). Irrigation with a high-potassium saline (saline 3A), however, resulted in the rapid development of a conduction block as described by Twarog & Roeder (1956). A subsequent recovery could be obtained by irrigating the ganglion for 2–5 min. in the normal physiological saline (Fig. 2).

The effects of removing the ventral portion of the nerve sheath from the fourth abdominal ganglion when irrigated with saline containing 70 mM./l. K (saline 2A) are illustrated in Fig. 3. With intact ganglia the complex action potential showed a progressive decline and approached a complete conduction block at between 20 and

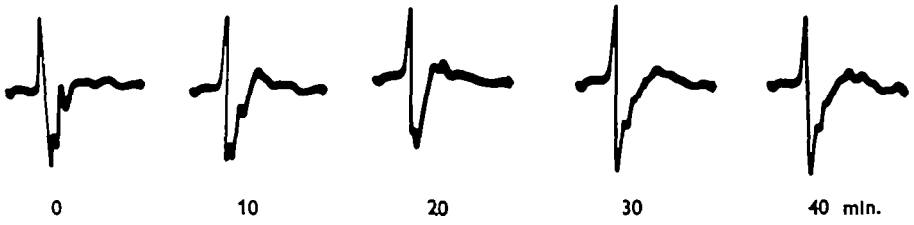

Fig. 1. Fourth abdominal ganglion (for preparation see text, p. 637). Action potentials. Intact ganglion irrigated with normal physiological saline (saline 1A).

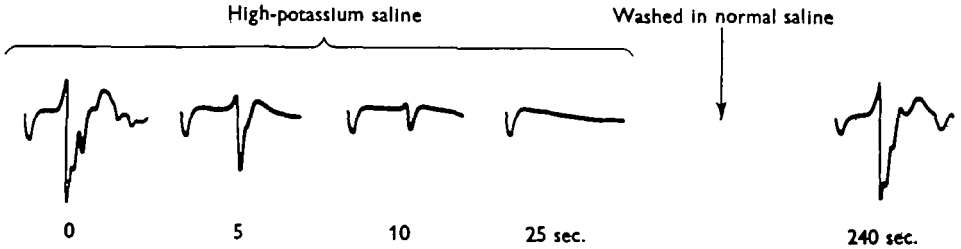

Fig. 2. Fourth abdominal ganglion. Action potentials. Intact ganglion irrigated with high-potassium saline (saline 3A). On the right, 240 sec., recovery after irrigation with normal saline.

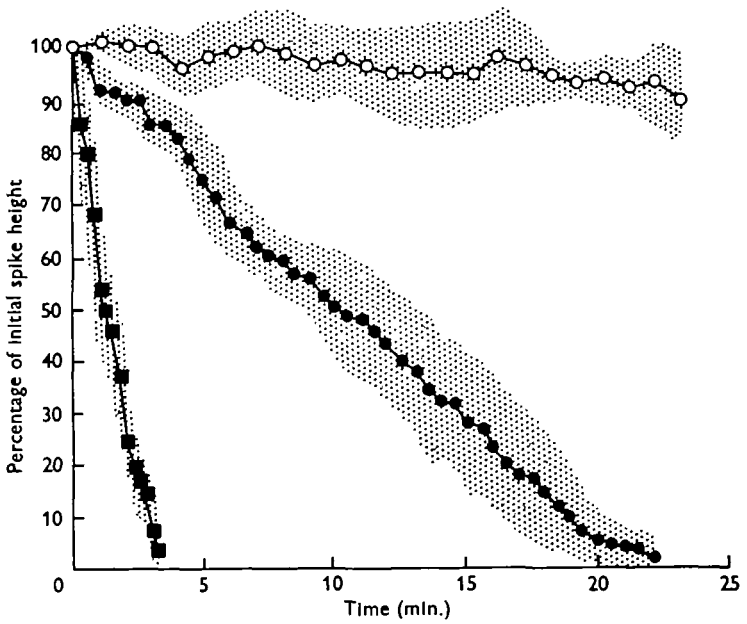

Fig. 3. Fourth abdominal ganglion. Intact ganglion irrigated with saline 1A, ○——○; intact ganglion irrigated with saline 2A, ●——●; desheathed ganglion irrigated with saline 2A, ■——■. The shaded areas represent the extent of twice the standard error of the means.

25 min. The desheathed preparation, on the other hand, showed a much more rapid decline in function and approached complete block to conduction in approximately 3–4 min.

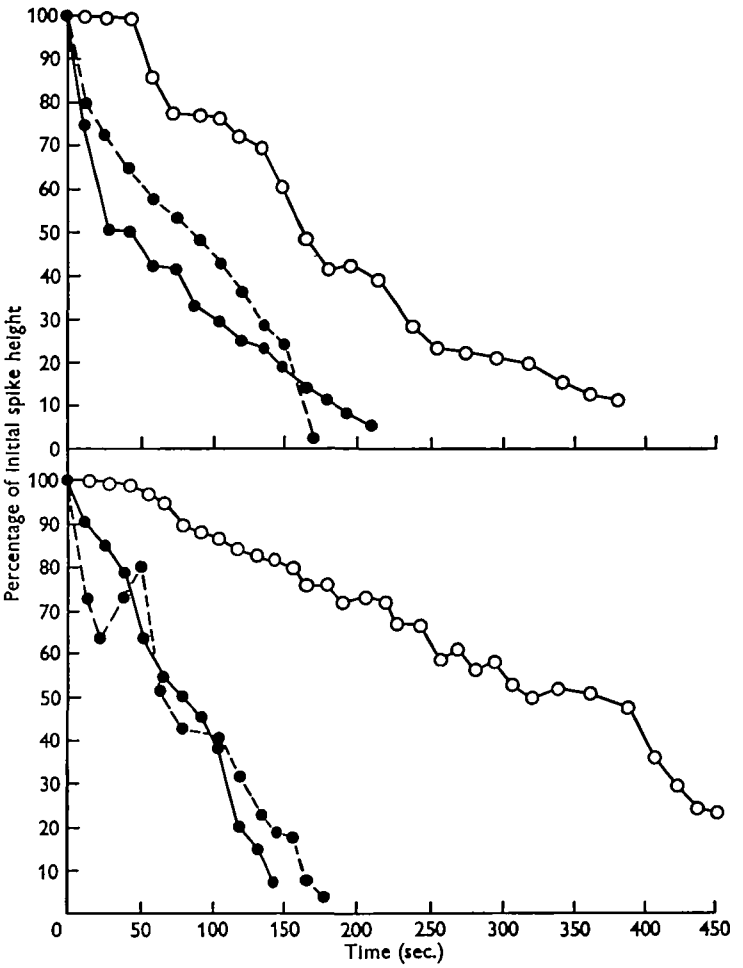

Fig. 4. Fourth abdominal ganglion. Two experiments involving desheathed ganglia, each irrigated with: (*a*) saline 2A, ●——●; (*b*) saline 1A, to restore activity; (*c*) saline 2B, ○——○; (*d*) saline 1A, to restore activity; (*e*) saline 2A, ●---●.

In the next group of experiments the rate of loss of conduction of desheathed ganglia irrigated with saline containing 70 mM./l. K (saline 2A) was compared with that obtained with a saline in which the cation concentrations were elevated to correspond to the levels in the extracellular fluid of the nerve cord in these conditions (saline 2B). In order to reduce the effects of individual variations the comparison of these two salines was in each case carried out on the same desheathed ganglion preparation. Fig. 4 shows the results of two typical experiments. The desheathed ganglia were first irrigated with the saline containing 70 mM./l. K which caused a fairly rapid loss of conduction. Following this the ganglia were irrigated in normal saline to restore the normal activity and were then irrigated with the saline containing the elevated catio

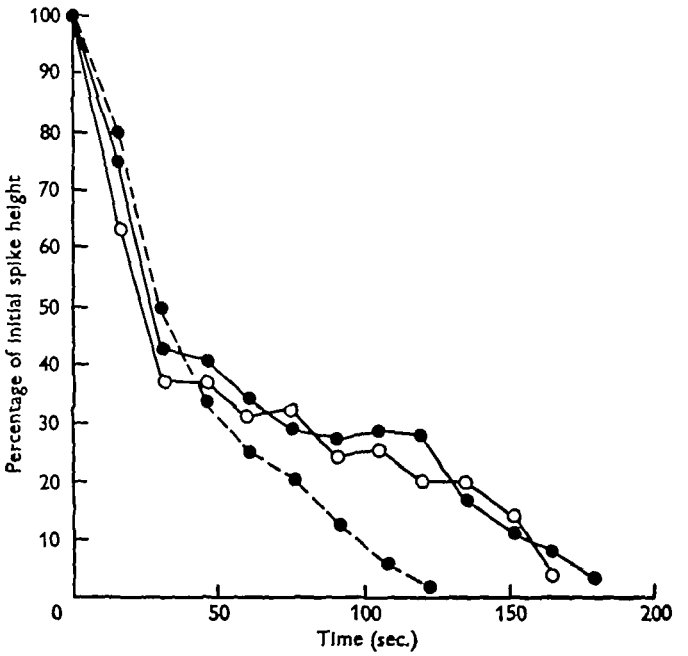

Fig. 5. Fourth abdominal ganglion, desheathed; irrigated with: (*a*) saline 2A, ●——●; (*b*) saline 1A, to restore activity; (*c*) saline 2C, ○——○; (*d*) saline 1A, to restore activity; (*e*) saline 2A, ●---●.

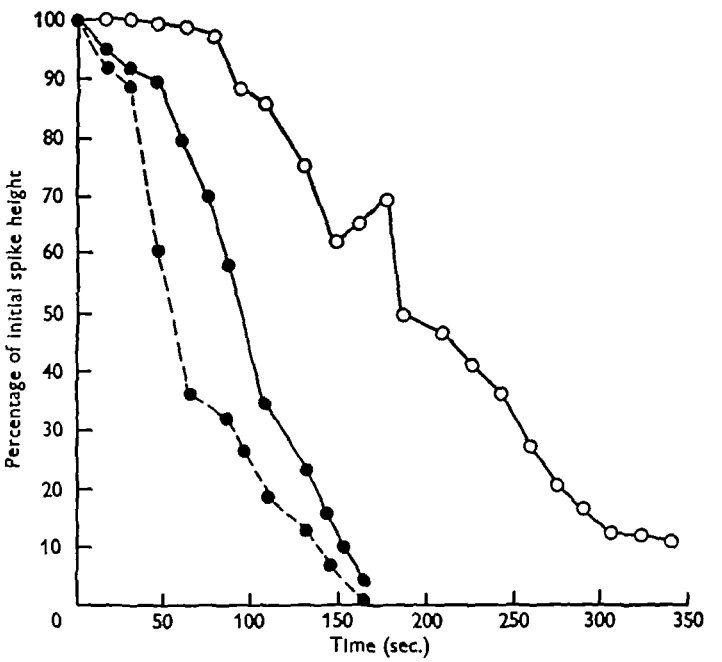

Fig. 6. Fourth abdominal ganglion, intact; irrigated with (*a*) saline 2A, ●——●; (*b*) saline 1A, to restore activity; (*c*) saline 2D; ○——○; (*d*) saline 1A, to restore activity; (*e*) saline 2A, ●---●.

concentrations (saline 2B). Finally the ganglia were irrigated once again in normal physiological saline before being irrigated with the original 70 mM./l. K saline. It is evident from these results that the decline in nervous conduction proceeded much more slowly in the saline containing the elevated cation levels even though the potassium concentration in this saline exceeded that of the 'basic' saline.

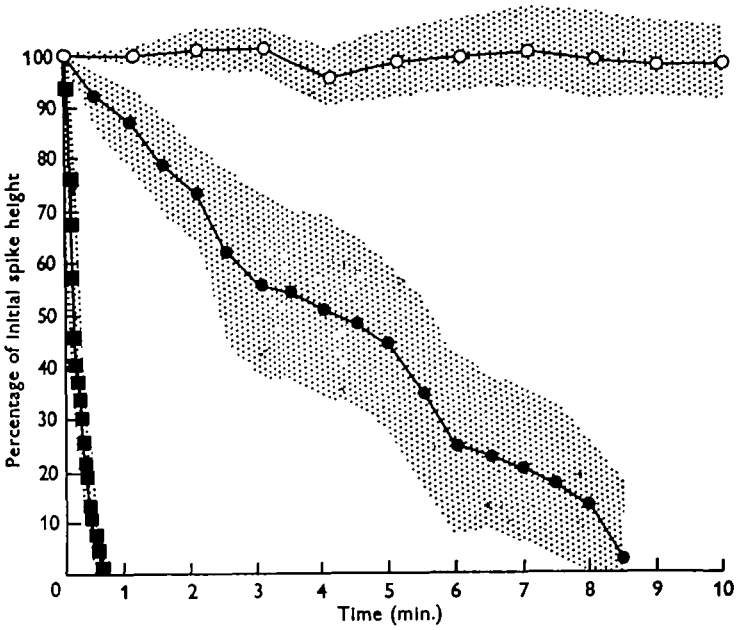

Fig. 7. Fourth abdominal ganglion. Intact ganglion irrigated with saline 1A, O——O; intact ganglion irrigated with saline 3A, ●——●; desheathed ganglion irrigated with saline 3A, ■——■. The shaded areas represent the extent of twice the standard error of the means.

To determine which of the ions was causing the delay in potassium depolarization described above, some further experiments were carried out in which the loss of excitability was compared as between salines in which only the sodium or calcium concentrations were elevated. Fig. 5 shows the effect of irrigating the desheathed ganglion with saline 2C (Table 1). It will be seen that there was very little difference in the rate of loss of conduction as compared with that obtained using the 'basic' saline. Irrigation with saline 2D, in which the sodium concentration was elevated to the extracellular level, resulted in a delayed decline in excitability (Fig. 6). These results indicate, therefore, that the slower rate of potassium depolarization obtained at the elevated cation levels must have been due to the increased concentration of sodium rather than of calcium ions.

Experiments were carried out using a saline in which the sodium and potassium concentrations were reversed (Table 1). The concentration of 154·8 mM./l. K (saline 3A) is of the same order as that in the high-potassium saline used by Twarog & Roeder (1956). Intact ganglia irrigated with this saline approached complete conduction block between 8 and 9 min.; desheathed ganglia, on the other hand, showed a more rapid loss of excitability and were effectively blocked within 1 min. (Fig. 7).

Treatment of desheathed fourth abdominal ganglia with elevated cation concentrations (saline 3 B) resulted in a slower loss of excitability as compared with the ion levels in the 'basic' saline (154·8 mM./l. K) (Fig. 8).

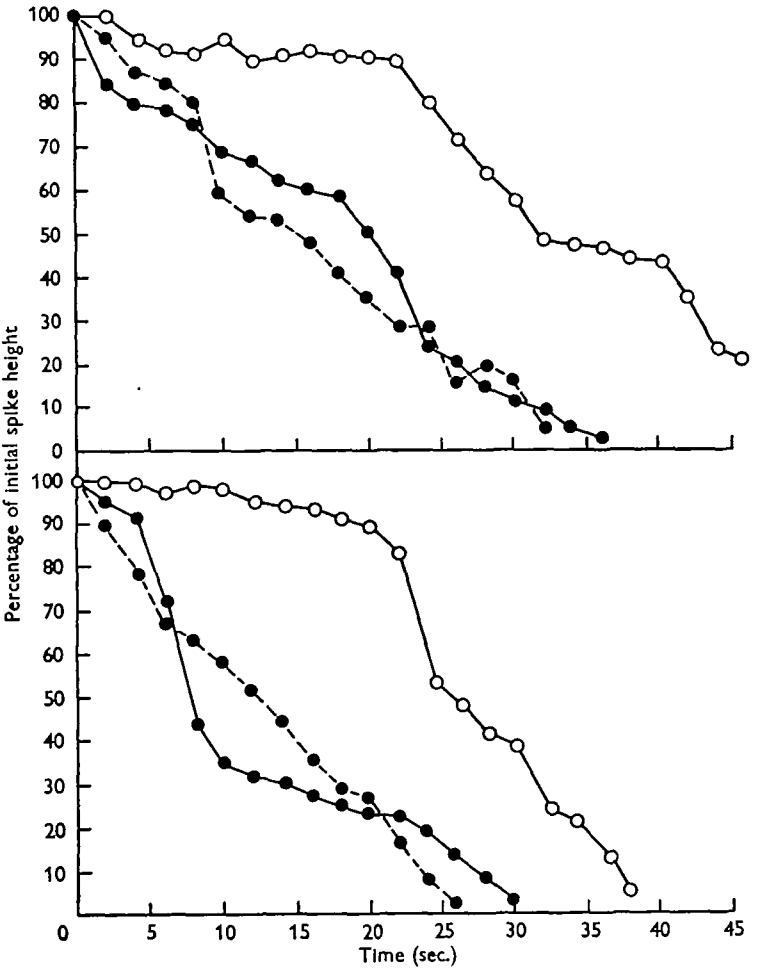

Fig. 8. Fourth abdominal ganglion. Two experiments, involving desheathed ganglia, each irrigated with: (*a*) saline 3 A, ●——●; (*b*) saline 1 A, to restore activity; (*c*) saline 3 B, O——O; (*d*) saline 1 A, to restore activity; (*e*) saline 3 A, ●---●.

Experiments on the intact terminal abdominal ganglion confirmed the observations of Twarog & Roeder (1956) that extremely high concentrations of acetylcholine (50 mM./l.) did not significantly alter the synaptic responses (Fig. 9 A). As previous authors have shown, desheathing the ganglia resulted in a rapid decrease in synaptic response at these levels of acetylcholine (Fig. 9 B). Alterations of the cation concentration to approximate to that of the extracellular fluid (saline 1 B) did not appear significantly to affect the decrease in synaptic response at high acetylcholine concentration (Fig. 9 C).

In injection experiments 100 mM./l. acetylcholine solution was introduced beneath the sheath surrounding the terminal abdominal ganglion. The smallest volume of fluid injected (0·03 μl.) was approximately equivalent to the extracellular space of the

ganglion (Treherne, 1962*b*) and with 100 mM./l. acetylcholine it was calculated to have produced an extracellular concentration corresponding to that of a desheathed ganglion in 50 mM./l. solution. It was found that injection of this volume of acetylcholine solution beneath the sheath did not produce the rapid loss of conduction resulting from the desheathing procedure (Fig 10B). Similarly, injection of 0·06 μl. of 100 mM./l. acetylcholine did not appear to block the synaptic conduction.

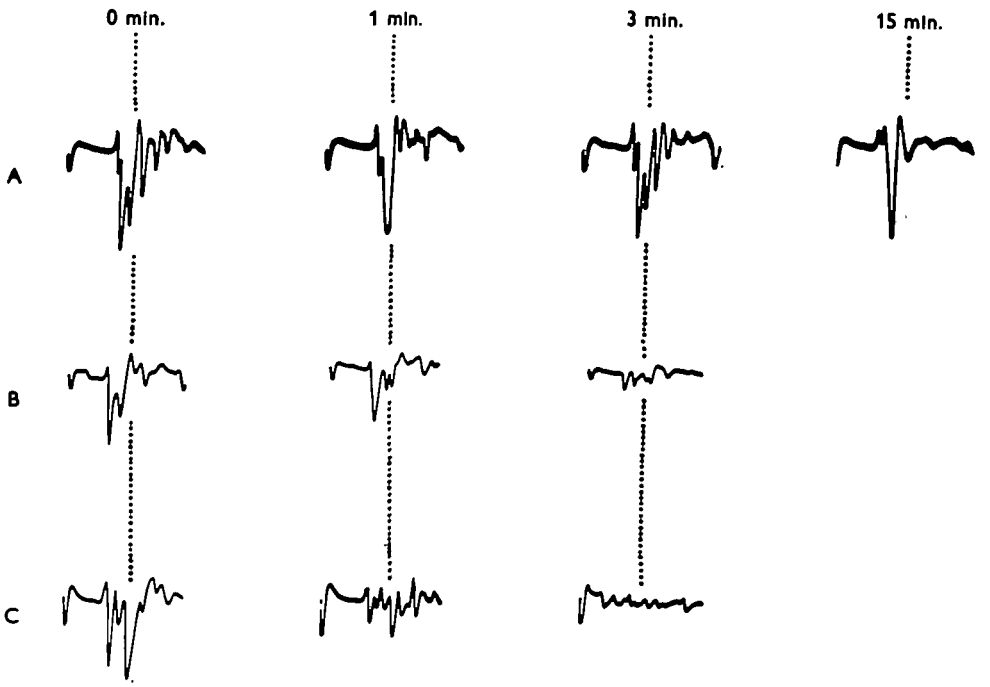

Fig. 9. Sixth abdominal ganglion (for preparation see text, p. 632). Action potentials: A, intact ganglion irrigated with 50 mM./l. acetylcholine in saline 1A; B, desheathed ganglion irrigated with 50 mM./l. acetylcholine in saline 1A; C, desheathed ganglion irrigated with 50 mM./l. acetylcholine in saline 1B.

DISCUSSION

The experiments described here confirm the observations of Hoyle (1953) on peripheral nerve of *Locusta*, and of Twarog & Roeder (1956) on *Periplaneta* nerve cord, that removal of the nerve sheath results in a rapid loss of excitability in high external concentrations of potassium ions. This effect has in the past been explained by assuming that the nerve sheath represents a significant diffusion barrier restricting the entry of ions and molecules into the underlying tissues. More recently it has been shown, however, that exchanges of ions appear to take place relatively rapidly between the haemolymph and the central nervous system of *Periplaneta* (Treherne, 1961*a*). It has also been demonstrated that removal of the nerve sheath involves the disruption of a Donnan equilibrium which has been shown to exist between the haemolymph and the extracellular fluid in the central nervous system (Treherne, 1962*a*, *b*). The extracellular levels of the monovalent cations were found to exceed those of the haemolymph by a factor of approximately 1·8, while the calcium concentration was 3·8 times greater than that of the outside medium. It was suggested on the basis of

these results that the enhanced rate of depolarization obtained in high concentrations of potassium ions in desheathed preparations might in part result from the changed ionic environment of the nerve cells. The present investigation has shown that with high external concentrations of potassium ions the substitution of salines containing cations at the extracellular level resulted in delayed development of conduction block in desheathed preparations. With the saline containing 70 mM./l. K the rate of loss of excitability rose to approximately half that of the intact preparation in the presence of the elevated cation levels. With the saline in which concentrations of sodium and

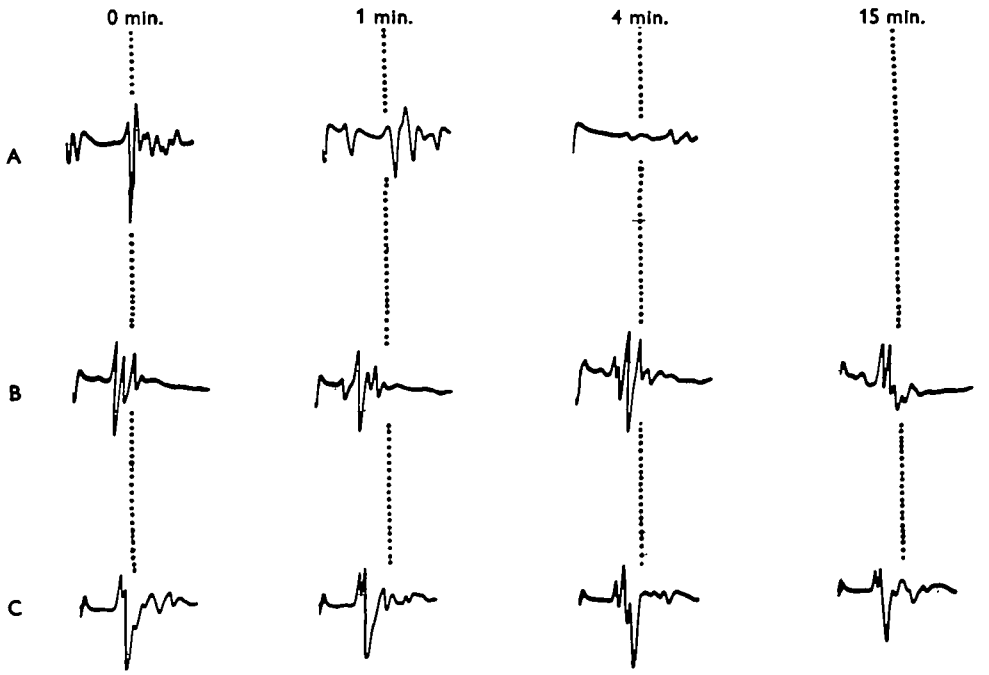

Fig. 10. Sixth abdominal ganglion. Action potentials: A, desheathed ganglion irrigated with 50 mM./l. acetylcholine in saline 1A; B, intact ganglion, 0·03 μl. of 100 mM./l. acetylcholine injected under nerve sheath; C, intact ganglion, 0·06 μl. of 100 mM./l. acetylcholine injected under nerve sheath.

potassium ions were reversed (154·8 mM./l. K) this delay accounted for a smaller fraction of the difference between the rates of loss of excitability in desheathed and intact preparations. As with frog nerve fibres (Lundberg, 1951) the delayed potassium depolarization in this insect appeared to be due to the excess of sodium ions in the experimental salines. The proportionally smaller delay in depolarization obtained with the high-potassium saline presumably resulted from reduced concentrations of sodium ions present in this saline.

Despite the effect which calcium ions are known to have on the level of excitability of the synaptic membranes (cf. Brink, 1954) substitution of salines containing elevated cation concentrations, corresponding to those in the extracellular fluid, did not significantly affect the rate of decrease in synaptic response in desheathed preparations subjected to massive concentrations of acetylcholine. In this case, then, the marked difference in the rate of conduction as between desheathed and intact terminal ganglia, in high concentrations of acetylcholine, cannot be attributed to the changed

extracellular ionic environment resulting from the disruption of the Donnan equilibrium in the desheathed preparation.

It is clear from the above considerations that in certain conditions a significant proportion of the effects resulting from the desheathing of portions of the central nervous system of this insect can be attributed to changes in the concentrations of the extracellular cations resulting from the disruption of the Donnan equilibrium with the haemolymph. However, at very high external potassium levels, when potassium ions replace sodium ions, this effect becomes relatively small.

Similarly, the rapid decrease in synaptic transmission in the desheathed terminal ganglion in the presence of high concentrations of acetylcholine appears not to be due to changes in the ionic composition of the extracellular fluid.

It follows from the above conclusions that some additional factors must be involved in producing the enhanced rates of depolarization obtained with desheathed preparations in 154·8 mM./l. K and in 50 mM./l. acetylcholine. As has already been pointed out the relatively rapid movement of ^{42}K measured across the nerve sheath of *Periplaneta* would seem to eliminate the possibility that this structure is functioning as a significant diffusion barrier (Treherne, 1961*a*, 1962*b*). No direct measurements have been made of the permeability of the nerve sheath to acetylcholine but the observation that synaptic conduction continues after injection beneath the sheath does not support the hypothesis that this structure drastically restricts entry of these molecules into the terminal abdominal ganglion. A similar state of affairs would seem to exist in the central nervous system of *Locusta*, for injection of high concentrations of acetylcholine beneath the nerve sheath has been shown to be without effect in this insect (Harlow, 1958).

The changed electrical responses in adverse chemical conditions in the absence of the nerve sheath may be due, in part at least, to factors such as changes in the concentrations of some unidentified chemical components in the extracellular fluid or to structural damage caused by the desheathing procedure. The possibility also exists that the enhanced rates of potassium depolarization obtained in desheathed preparations might result from the organization of the extracellular space in the cockroach ganglion. As has been pointed out by Smith & Treherne (1963) the extracellular system consists of relatively large spaces towards the periphery together with a ramifying network of exceedingly fine channels in the deeper layers of the ganglion. The peripheral spaces, which include the glial lacunar system of Wigglesworth (1960), therefore contain the greater part of the extracellular fluid. Thus the extremely rapid exchanges of ions and molecules between the extracellular fluid and the haemolymph described by Treherne (1961*b*, 1962*b*) may be those associated with the large peripheral extracellular spaces and might effectively mask any slower exchanges taking place with the deeper layers of the ganglion. The desheathing of the terminal abdominal ganglion of the *Periplaneta* is known to involve a significant increase in the volume of the extracellular fluid (Treherne 1962*b*). It is conceivable that this increase might be reflected in some enlargement of any very restricted extracellular spaces so as to increase the accessibility of ions to these regions of the ganglion. Thus in the present experiments it could be postulated that the rapid development of conduction block in the absence of the nerve sheath in saline containing 70 mM./l. K resulted in part from the changed ion concentrations in the extracellular fluid, caused by the disruption of

the Donnan equilibrium with the haemolymph, and to some additional factors such as the increased accessibility to ions due to enlargements of the restricted extracellular spaces in the deeper layers of the ganglion. With extremely high potassium and correspondingly low sodium levels, on the other hand, the effects of the changed ionic environment surrounding the nerve cells were relatively small and depolarization appeared to be due to the other changes resulting from the desheathing procedure.

SUMMARY

1. Irrigation of abdominal ganglia of *Periplaneta americana* with salines containing excess potassium ions resulted in the development of a reversible conduction block. Removal of portions of the cellular and fibrous nerve sheath produced an accelerated potassium depolarization as described by Twarog & Roeder (1956).

2. Elevation of the cation concentrations to correspond to the extracellular levels resulted in delayed conduction block in desheathed preparations irrigated with high-potassium saline. At 70 mM./l. K the rate of depolarization was delayed to approximately half that of intact ganglia. At extremely high potassium concentrations this effect became relatively small.

3. It is suggested that the enhanced rates of potassium depolarization obtained in desheathed preparations partly result from the changed ionic composition of the extracellular fluid resulting from the desheathing procedure. The possible additional factors involved in the desheathing procedure are discussed.

4. 50 mM./l. acetylcholine had little effect on the synaptic transmission in intact terminal abdominal ganglia but caused rapid loss of conduction in desheathed preparations. Injection of equivalent amounts of acetylcholine beneath the nerve sheath did not cause rapid conduction block, and it is suggested that the effects of desheathing are not necessarily caused by the removal of a relatively impermeable superficial diffusion barrier.

I am indebted to Miss A. Fielden and Dr H. Fraser Rowell for their help during the course of this work.

REFERENCES

BRINK, F. (1954). The role of calcium ions in neural processes. *Pharm. Rev.* **6**, 243–98.

HARLOW, P. A. (1958). The action of drugs on the nervous system of the locust (*Locusta migratoria*). *Ann. Appl. Biol.* **46**, 55–73.

HOYLE, G. (1953). Potassium ions and insect nerve muscle. *J. Exp. Biol.* **30**, 121–35.

LUNDBERG, A. (1951). On the ability of some cations to inhibit the potassium depolarization of frog nerve fibres. *Acta physiol. scand.* **22**, 365–75.

SMITH, D. S. & TREHERNE, J. E. (1963). Functional aspects of the organization of the insect nervous system. *Adv. Ins. Physiol.* **1** (eds. J. W. L. Beament, J. E. Treherne & V. B. Wigglesworth). London: Academic Press. (In the Press.)

TREHERNE, J. E. (1961*a*). Sodium and potassium fluxes in the abdominal nerve cord of the cockroach, *Periplaneta americana* L. *J. Exp. Biol.* **38**, 315–22.

TREHERNE, J. E. (1961*b*). The kinetics of sodium transfer in the central nervous system of the cockroach, *Periplaneta americana* L. *J. Exp. Biol.* **38**, 737–46.

TREHERNE, J. E. (1962*a*). Distribution of water and inorganic ions in the central nervous system of an insect (*Periplaneta americana* L.). *Nature, Lond.*, **193**, 750–2.

TREHERNE, J. E. (1962*b*). The distribution and exchange of some ions and molecules in the central nervous system of *Periplaneta americana* L. *J. Exp. Biol.* **39**, 193–217.

TWAROG, B. M. & ROEDER, K. D. (1956). Properties of the connective tissue sheath of the cockroach abdominal nerve cord. *Biol. Bull., Woods Hole*, **111**, 278–86.

WIGGLESWORTH, V. B. (1960). The nutrition of the central nervous system in the cockroach, *Periplaneta americana* L. The role of perineurium and glial cells in the mobilization of reserves. *J. Exp. Biol.* **37**. 500–12.