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# IONIC COMPOSITION OF HAEMOLYMPH AND NERVOUS FUNCTION IN THE COCKROACH, *PERIPLANETA AMERICANA* L.

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## INTRODUCTION

The ionic composition of the haemolymph of the American cockroach, *Periplaneta americana*, has been fully studied during the past 20 years (Tobias, 1948; Van Asperen & Van Esch, 1956; Treherne, 1961; Pichon, 1963; Pichon & Boistel, 1963*a*, *b*; Brady, 1967*a*, *b*). It has been shown that whereas the sodium concentration is higher than that of potassium, thus resembling the blood of vertebrates, the potassium concentration may vary widely; the potassium level of the haemolymph increases, for example, when the insect is fed on a leaf diet (Tobias, 1948; Pichon & Boistel, 1963*b*) or submitted to prolonged starvation and dehydratation (Pichon & Boistel, 1963*a*) and may reach or even exceed 50 mM/l. Moreover, extremely large variations of the Na/K ratio have been recorded between different insects reared in apparently identical conditions (Pichon, 1963).

It is reasonable to suppose that such variations may appreciably modify the excitability of the neurones. The effects of inorganic ions on insect nerve fibres have been indeed thoroughly investigated using the microelectrode technique (see Yamasaki & Narahashi, 1959; Boistel, 1960; Narahashi & Yamasaki, 1960; Narahashi, 1966) and it has been concluded that the sodium theory was essentially valid for the giant axons of the cockroach. According to the curves given by Yamasaki & Narahashi (1959) the giant axons would be strongly depolarized in potassium concentrations such as those which have been measured in the haemolymph. Roeder (1948) found, however, that spontaneous activity and synaptic transmission in the isolated nerve cord of the cockroach were not immediately modified by variations in potassium concentrations in the medium between 0 and 50 mM/l. $K^+$. More recently, we have shown that perfusion of the last abdominal ganglion of the cockroach with a saline containing 52 mM/l. $K^+$ induced a transient increase in the 'spontaneous' activity recorded at the level of the abdominal nerve cord; this increase was followed by a blockage. The delay between the beginning of the perfusion and the maximum of activity is greatly reduced in de-sheathed preparations (Pichon & Boistel, 1965). This fact is in good agreement with observations by Twarog & Roeder (1956) that when an intact segment of the abdominal nerve cord is bathed in 180 mM-KCl it takes 12–18 min. for conduction to be blocked, whereas this delay is only 10 sec. if a desheathed segment is perfused with this same saline. These authors concluded that the nerve sheath acts as a diffusion barrier against ions. Treherne (1961, 1962*a–c*) has shown, however, that the nerve sheath is freely permeable to ions and that, rather than a static impermeability, a dynamic steady state can exist between haemolymph and

the extracellular fluid. Removal of the nerve sheath would result in drastic changes in the composition of this extracellular fluid due to the disruption of a Donnan equilibrium with the haemolymph. These alterations would be responsible for the enhanced rates of depolarization observed in de-sheathed preparations. It has in fact been shown that elevation of the cation concentration to the extracellular levels resulted in a delayed conduction block in de-sheathed nerve cords of the cockroach irrigated with high potassium saline (Treherne, 1962*c*). In a similar way it has been shown that, whereas a saline containing 12·3 mM-$K^+$ appreciably depolarized the giant axons of the cockroach in a de-sheathed nerve cord, a saline containing 17·1 mM-$K^+$ did not alter significantly the resting potential provided the cation concentrations were elevated to the extracellular level (358·2 mM-$Na^+$, 17·3 mM-$Ca^{2+}$; Pichon & Boistel, 1967).

A new approach to this problem has been possible with the use of fine-tipped and mechanically resistant microelectrodes which allowed reliable intracellular recordings of membrane potentials to be made in sheathed nerve cords (Pichon & Boistel, 1966). Experiments carried out on isolated nerve cords bathed in different salines have given the following results:

The resting potentials were lower and the action potentials higher in sheathed preparations than in de-sheathed ones.

A saline containing 12·3 mM-$K^+$ (instead of 3·1) produced only slight depolarization of the giant axons in sheathed preparations, leaving the action potential unchanged, whereas in de-sheathed preparations the resting potentials were decreased by some 10 mV. and the action potentials reduced by more than a half, thus demonstrating once more the disturbances involved by the de-sheathing procedure.

From the above results, it is logical to think that in the intact nerve cord, bathed in insect's own haemolymph, the nerve fibres may have rather low resting potentials associated with high action potentials. It should be noted, however, that there may be a certain discrepancy between *in vivo* membrane potentials and those recorded *in vitro*. In the first place errors may arise from the nature of physiological salines used for electrophysiological investigations and which differ considerably from the haemolymph. Moreover, in Ringer solutions, all the cations are free, whereas part of them, for instance $K^+$ or $Ca^{2+}$ ions, may be bound to organic molecules in the haemolymph. In addition, it has been recently shown that an appreciable fraction of the haemolymph $K^+$ might be related to the haemocytes and thus be inactive for excitable membranes (Brady, 1967*a*, *b*). Another source of error may arise from the more or less extended lesion of the sheath and of the tracheae which necessarily occurs when the nerve cord is dissected away.

We shall describe in this paper the results of our investigations on intact nerve cords bathed in insect's own haemolymph. They will concern the *in situ* values of the membrane potentials of the giant axons.

## MATERIAL AND METHODS

### *Recording apparatus*

The same technique as that previously described (Pichon & Boistel, 1967) has been used throughout the present experiments, except for the reference electrode which consisted here of a hooked Ag–AgCl electrode held by a micromanipulator.

### *Solutions*

Except for long-term experiments, in which normal or 'H' salines (Pichon & Boistel, 1967) were used, no solution was employed in this work, the nerve cord being bathed in the insect's blood.

### *Experimental procedure*

Two different procedures were used:

In the first the insect was pinned ventral side upwards on a piece of cork. The animal was immobilized with plasticine and a small window was cut in the cuticle and also in the underlying epidermis just above the nerve cord between the 3rd and the 5th abdominal ganglia. A pair of hooked Ag–AgCl electrodes held on a micromanipulator were gently pushed beneath the connectives and lifted up in order to stretch the nerve cord slightly. One of these electrodes was connected to ground and was used as an indifferent electrode. The microelectrode was introduced into a giant axon between these two electrodes, which were also used to record extracellularly the activity of the nerve fibres. The cerci were stimulated by a puff of air and the corresponding activity in the nerve cord was continuously recorded by means of a kymograph camera (Grass C4 Camera). The advantage of this procedure is that it limits the area of injury. Unfortunately, such a preparation cannot be used for long-term experiments because of the very fast desiccation of the nerve cord on the electrodes and the frequent contractions of the abdomen of the insect which soon break the microelectrode tip.

The second procedure involved more extensive dissection but allowed prolonged intracellular recordings to be carried out. The head, legs and wings were cut away and the insect was pinned, dorsal side uppermost, on a piece of cork. A large window was cut in the dorsal integument and the underlying digestive duct was gently pushed aside by means of the indifferent electrode until the nerve cord was made visible. The two stimulating electrodes where placed under the connectives between the 3rd and the 4th ganglia and the microelectrode was introduced between the 2nd and the 3rd ganglia into a giant axon.

Experiments were carried out at room temperature ranging from 20 to 26° C. on adult male cockroaches reared in the laboratory on a mixed diet (lettuce, wheat flour, milk powder and tap water).

## RESULTS

### *Membrane potentials of giant axons in nerve cords bathed in haemolymph*

The magnitude of the action potential, resting potential, 'sheath potential',* overshoot and also the rates of rise and fall of the action potential of giant axons have been measured in intact nerve cords bathed in the insect's own haemolymph (Table 1). Typical tracings of the action potential and its derivative are given in Fig. 1. It is seen that very low resting potentials ($43{\cdot}0 \pm 4{\cdot}8$ mV.) were associated with large action potentials ($105{\cdot}1 \pm 6{\cdot}8$ mV.), thus giving a very high overshoot of $62{\cdot}1 \pm 7{\cdot}0$ mV.

* When a microelectrode was withdrawn slowly enough from a giant axon, two successive potential changes were recorded: a positive deflexion often followed by a rapid negative deflexion to the original zero level. It has been agreed to call 'sheath potential' this last deflexion which seemed to be most generally associated with the nerve sheath, and 'resting potential' the potential difference between inside of a giant axon and zero potential. (Pichon & Boistel, 1967).

The 'sheath potential' reached 28·2 ± 3·9 mV. The mean maximum rates of rise and fall were respectively of 1161 ± 168 V./sec. and 386 ± 65 V./sec. (Table 1).

Table 1. *Membrane potentials of giant axons of* Periplaneta *recorded* in situ

| | | | | | | Maximum rate | |
|---|---|---|---|---|---|---|---|
| $t°$ | $n°$ | Action potential (mV.) | Resting potential (mV.) | 'Sheath potential' (mV.) | Over-shoot (mV.) | Of rise (V./sec.) | Of fall (V./sec.) |
| 23° | 1 | 112 | 40 | — | 72 | — | — |
| | 2 | 112 | 43 | — | 69 | 1200 | 420 |
| | 3 | 95 | 35 | — | 60 | 1000 | 340 |
| 26° | 4 | 100 | 44 | — | 56 | 950 | 410 |
| | 5 | 95 | 43 | — | 52 | 1050 | 340 |
| | 6 | 102 | 44 | — | 58 | 1050 | 340 |
| | 7 | 110 | 50 | 22 | 60 | 1420 | 520 |
| | 8 | 105 | 50 | 32 | 55 | 1270 | 410 |
| | 9 | 100 | 38 | 27 | 62 | 1130 | 340 |
| | 10 | 110 | 49 | 30 | 61 | 1380 | 450 |
| 20·5° | 11 | 115 | 40 | — | 75 | — | — |
| | 12 | 105 | 40 | 30 | 65 | — | — |
| Mean | | 105·1 | 43·0 | 28·2 | 62·1 | 1161 | 386 |

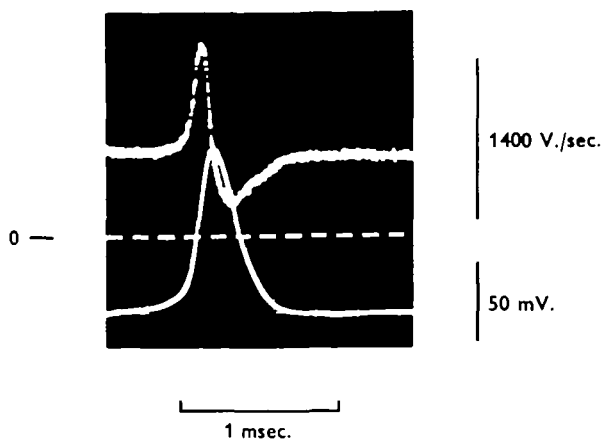


Fig. 1. *In situ* recording of an action potential (lower tracing) and its derivative (upper tracing). Interrupted line indicates zero potential. 26° C.

## DISCUSSION

The preceding results have shown that membrane potentials of the giant axons of the cockroach measured *in situ*, in nerve cords bathed in the insect's own haemolymph, are very different from those reported for de-sheathed preparations studied *in vitro* in Ringer solution (Yamasaki & Narahashi, 1959; Boistel, 1960; Narahashi & Yamasaki, 1960), for a mean resting potential of 43·0 mV. is associated with large action potentials (105·1 mV.) so as to give a proportionally large overshoot of 62·1 mV. These results are in agreement with previously reported experiments on sheathed nerve cords studied *in vitro* (Pichon & Boistel, 1967). The interpretation of the previous results is, however, not altogether clear at the time: two different explanations might

be valuable which differ essentially by the signification given to the 'sheath potential'.

The simplest explanation is that the true resting potential (RP) of the giant axons recorded in sheathed nerve cords would correspond to the algebraic difference between the measured resting potential (RPm) and the measured 'sheath potential' (SP). Thus for the present experiments:

$$[RP] = [RPm] - [SP]$$
$$= (43{\cdot}0) - (-28{\cdot}2) = 43{\cdot}0 + 28{\cdot}2 = 71{\cdot}2 \text{ mV.}$$

This value is somewhat higher than that in de-sheathed nerve cords bathed in Ringer solution (67·4 mV., Pichon & Boistel, 1967), but is still lower than the value of 77 mV. reported by Narahashi & Yamasaki (1960) for the same preparation. This corrected value of the resting potential would correspond to a mean overshoot of 33·9 mV. The latter value, which is appreciably higher than that reported for de-sheathed preparations bathed in Ringer solution (18·5 mV., Pichon & Boistel, 1967; 22 mV., Narahashi & Yamasaki, 1960), is consistent with an increased sodium concentration in the extracellular fluid suggested by Treherne (1962*b*).

This explanation which seems at first quite satisfactory is unconfirmed for at least two reasons. First, it is difficult to concile this corrected value of the resting potential with a high extracellular $K^+$ concentration demonstrated by Treherne (1962*b*). It has been shown, for example, that the resting potential of the cockroach giant axons depends upon external $K^+$ concentrations (Yamasaki & Narahashi, 1959). According to the curve given by the latter authors a resting potential of 71·2 mV. would correspond to an external $K^+$ concentration lower than 4 mM/l. Now the estimated extracellular concentration in the sheathed nerve cord of *Periplaneta* is as high as 17·1 mM (Treherne, 1962*b*).* The second reason is that the 'sheath potential' may be an artifact determined by variations of the microelectrode tip potential in different ionic solution (Picton & Boistel, 1967). This latter explanation must not be definitively discarded for no further information concerning the nature of this 'sheath potential' is available, and new experiments, including the study of the isolated nerve sheath, are needed.†

The observed values of the resting and action potentials in sheathed nerve cords may also be interpreted in terms of elevated cations levels in the extracellular fluid. This explanation appeared to be satisfactory in the case of sheathed nerve cords studied *in vitro* (Pichon & Boistel, 1967) and may be at least partly applicable in the present experiments. If we assume, therefore, that the resting potential is mainly determined by the external $K^+$ concentration, according to the curves given by Yamasaki & Narahashi (1959), then a resting potential of 43·0 mV. would correspond to about 28 mM-$K^+$ in the extra-axonal fluid. Taking a $K^+$ haemolymph/$K^+$ extracellular ratio

* Experiments carried out using 'extracellular like' saline have shown that the resting potential of the giant axons of *Periplaneta* recorded in de-sheathed preparations may be as high as 64·6 mV. when the preparation is bathed in a saline containing 17·1 mM-$K^+$ provided that $Na^+$ and $Ca^{2+}$ concentrations were raised to the extracellular level (Pichon & Boistel, 1967). These experiments may, nevertheless, be unreliable because of the great difference which exists between the chemical composition of the extracellular fluid and that of 'extracellular like' salines, especially concerning Cl ions. Furthermore, the possibility exists that chemical activity of extracellular cations may be reduced in the presence of indiffusible extracellular anions (cf. Treherne, 1967).

† Moreover, a possibility remains that, an important fraction of the whole blood potassium being sequestered within the haemocytes (Brady, 1967*b*), the actual potassium concentration in the extracellular fluid might be notably lower than the estimated one from experiments on isolated nerve cords bathing in a saline containing as much as 12·5 mM/l. of potassium.

of 0·71 (Treherne, 1962*b*), the corresponding $K^+$ concentration in the haemolymph would be of about 20 mM. This value is only slightly lower than the measured one (24·0 ± 1·8 mV., Pichon, 1963). The fact that the membrane may retain its excitability in spite of an appreciable depolarization may be related to the high extracellular $Ca^{2+}$ level. Narahashi (1966) has therefore demonstrated that the increase of the $K^+$ concentration from the normal one (3·1 mM) to 40 mM depolarized the membrane of the giant axon of the cockroach and blocked the action potential in presence of normal 1·8 mM $Ca^{2+}$ whereas, when the $Ca^{2+}$ concentration was raised to 54 mM, the same high $K^+$ concentration resulted in a depolarization while the maximum rate of rise of the action potential decreased only to a small extent. It seems, moreover, possible that $Mg^{2+}$ ions, which are contained in the haemolymph in relatively large amounts, may be concentrated in the extracellular spaces of the nerve cord of *Periplaneta* as for *Carausius* (Treherne, 1965*a*, *b*) and that the stabilizing action of these ions (also demonstrated by Narahashi, 1966) may add to that of $Ca^{2+}$ ions. If the action potential of the giant axons of *Periplaneta* behaves as a sodium electrode, the overshoot may approach the value of the equilibrium potential for sodium which is given by the following equation:

$$E_{Na} = \frac{RT}{F} \log_e \frac{Na_e}{Na_i}, \qquad (1)$$

where $E_{Na}$ is the sodium equilibrium potential, $R$ is the gas constant, $T$ the absolute temperature, $F$ the Faraday, $Na_e$ and $Na_i$ the sodium activities respectively in the extracellular fluid and inside the nerve cells. Using the values of 283·6 and 67·2 mM for $Na_e$ and $Na_i$ (Treherne, 1965*a*), $E_{Na}$ would be equivalent to 37·2 mV. This value is clearly lower than the recorded overshoot, the discrepancy between the observed value (62·1 mV.) and the calculated one amounting to 24·9 mV. Since divalent cations such as $Ca^{2+}$ contribute little to the inward membrane current during the rising phase of the action potential as suggested by Narahashi (1966), a higher value of $E_{Na}$ must account for this difference. It seems likely that the sodium concentration in cellular components of the abdominal nerve cord may be lower *in situ* than *in vitro*. It is known, for example, that resting *Sepia* axons kept at about 10° C. gained sodium at a rate of 8·8 mM/kg. axon/hr. (Keynes & Lewis, 1951). The value of $Na^+$concentration in the axoplasm of the giant axons of *Periplaneta* may be calculated using equation (1), thus keeping the same value for $Na_e$ and taking $E_{Na}$ = 62·1 mV. (i.e. the overshoot value), $Na_i$ would be 25·6 mM/l. of axoplasm water. This low value agrees quite well with the extrapolated $Na^+$ concentration of about 25 mM/l. of axoplasm in freshly dissected axons of *Sepia* (Keynes & Lewis, 1951). A possible higher $Na^+$ concentration in the extracellular spaces of *in situ* nerve cords of *Periplaneta* (resulting also in a higher $E_{Na}$) is also to be expected. When the nerve cord is dissected away—indeed, whenever the two ends of the preparation are ligatured and the nerve sheath kept intact—an exchange between the outside saline and the extracellular fluid may be possible by the cut ends of the segmental nerves. This exchange could result in an increase of the volume of the extracellular fluid and in a decrease in the extracellular cation concentration.* This increase in the volume of the extracellular fluid in excised

* We have therefore suggested that such diffusion process occurring at the level of the sectioned cercal nerves might be responsible, for example, for the observed shortening of the delay separating the application of a high $K^+$ saline to the sixth abdominal ganglion of the cockroach from the maximum of activity of the giant axons (Pichon & Boistel, 1965).

nerve cords of *Periplaneta* would be consistent with the observations by Treherne (1965*b*) that the figure for the inulin space was higher for *Carausius* nerve cords soaked *in vitro* in the radioactive solution than for nerve cords perfused *in vivo* with this same solution.

The sodium equilibrium potential may thus be elevated *in situ* by two means: first, because of a low intracellular $Na^+$ concentration (resulting from a better functioning of the sodium pump) and, secondly, because of a higher extracellular $Na^+$ concentration related to the integrity of the nerve sheath. In these conditions, the occurrence of large overshoots in our experiments is quite explicable without postulating any possible error in the resting membrane potential measurement induced by the existence of the 'sheath potential'. Moreover, the large value of this 'sheath potential' in the present experiments (28·2 mV.) may be easily related to an enhanced difference between the ionic compositions of the extracellular fluid and the haemolymph in nerve cords kept *in situ*.

The value of 1161 and 386 V./sec. for the maximum rates of rise and fall of the action potential are very near the values of 1100 and 380 V./sec. reported by Yamasaki & Narahashi (1959) for de-sheathed nerve cords of *Periplaneta* bathed in Ringer solution. It therefore seems likely that the net ionic inward current during the rising phase of the action potential and the net ionic outward current during the falling phase of the action potential are equivalent, whether the mean resting potential was 43·0 mV. in intact preparations or 70·3 mV. in de-sheathed ones.

It is thus demonstrated that the nervous system of the cockroach may function quite satisfactorily despite a relatively high $K^+$ concentration in the haemolymph. This phenomenon seems at the time to be related to the specialized ionic composition of the extracellular fluid rather than to special properties of the giant axons.

## SUMMARY

1. Resting and action potentials have been recorded in giant axons of the cockroach when the intact nerve cord was bathed in the insect's own haemolymph.

2. Low resting potentials ($43·0 \pm 4·8$ mV.) and large action potentials ($105·1 \pm 6·8$ mV.) were obtained in these preparations as compared with those recorded in de-sheathed nerve cords.

3. Recordings of the maximum rates of rise and fall have shown that the shape of the action potential was essentially similar in de-sheathed preparations and in intact nerve cords.

4. These results have been discussed in terms of the unequal distribution of ions between the haemolymph, the extracellular fluid and the axoplasm of the giant axons.

5. The low measured resting potential agrees with a $K^+$ concentration in the haemolymph of about 20 mM./l., a value which is only slightly lower than the measured one (Pichon, 1963).

6. The occurrence of large action potentials in these apparently depolarized axons may be related to the stabilizing action of divalent cations such as $Ca^{2+}$ which are contained in the extracellular fluid in relatively large amounts.

7. The very large recorded overshoots ($62·1 \pm 7·0$ mV.) may be linked with a low sodium concentration in the axoplasm and a high sodium concentration in the extra-

cellular fluid of the giant axons of intact nerve cords, thus resulting in a high sodium equilibrium potential, $E_{Na}$.

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