SODIUM AND POTASSIUM FLUXES IN THE ABDOMINAL NERVE CORD OF THE COCKROACH, *PERIPLANETA AMERICANA* L.

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**INTRODUCTION**

The experiments of Hoyle (1953) on the effects of potassium on the electrical behaviour of locust peripheral nerve led to the suggestion that the continuous sheath which surrounded the nervous system of this insect functioned as a diffusion barrier restricting the entry of potassium ions into the underlying nerve cells. The subsequent investigation of Twarog & Roeder (1956) on the abdominal nerve cord of the cockroach confirmed the observations of Hoyle and showed that the presence of the cellular and fibrous sheath, or perilemma, apparently protected the central nervous system of this insect from the adverse effects of fluctuations in the ionic content of the haemolymph. It was also found that the perilemma restricted the entry of acetylcholine molecules into the central nervous system.

The presence of a relatively impermeable diffusion barrier around the central nervous system made it difficult to appreciate how the necessary exchanges of nutritive and excretory substances could take place between the haemolymph and the nervous tissues. In an investigation on the metabolism of some $^{14}$C-labelled compounds in the abdominal nerve cord of *Periplaneta americana* L. it was found, nevertheless, that there was a rather rapid influx of the relatively large glucose and trehalose molecules through the perilemma (Treherne, 1960). In an attempt to resolve this apparently paradoxical situation the present investigation was undertaken to determine the rates of influx of sodium and potassium ions into the abdominal nerve cord of *P. americana* L.

In this paper the nomenclature of Scharrer (1939) has been used to describe the layers in the sheath of the cockroach central nervous system. Accordingly the sheath, or perilemma, is divided into two parts: the outer fibrous neural lamella and the inner cellular perineurium.

**METHODS**

The adult male *P. americana* L. was used exclusively in these experiments as it possesses a nerve cord which is relatively free from associated fat body.

The penetration of sodium and potassium ions into the abdominal nerve cord was studied after the injection of $^{24}$Na and $^{42}$K into the haemolymph of this insect. The radioactive ions were incorporated, as NaCl and KCl, in an experimental solution in which the major chemical components approximated to those of cockroach haemolymph (Table 3). To obtain a satisfactory level of activity in the nervous tissue it was necessary to inject $100 \mu l$ of the radioactive solution which represented an appreciable
increase in haemolymph volume. Experiments in which smaller volumes were injected did not, however, differ significantly from those in which 100 µl. were used and it is assumed that the increase in haemolymph volume did not affect the validity of these results.

In each experiment at varying times after the injection of $^{24}$Na and $^{42}$K individual cockroaches were decapitated and the abdominal nerve cord was removed by dissection from the dorsal surface. The isolated radioactive nerve cords were quickly washed in isotonic dextrose solution, dried on filter-paper and then weighed on a torsion balance. Haemolymph samples were collected using silicone-lined glass pipettes. The radioactivity in the haemolymph and tissue was determined using an end-window counter (Mullard MX 123).

The sodium and potassium concentrations of the nerve cord and haemolymph were measured by means of an EEL flame photometer, the tissue samples being previously ashed in a muffle furnace on pieces of platinum foil at temperatures of 460–480°C.

The freezing-point depressions of haemolymph and experimental solutions were measured by the method of Ramsay (1949) as modified by Ramsay & Brown (1955).

**RESULTS**

The measurements on the sodium and potassium concentrations of the haemolymph and the abdominal nerve cord are tabulated in Table 1. The results for the tissue concentrations are, despite the similarity in age, sex and diet of the individuals used, characterized by a rather high degree of individual variation. These data are similar in this respect to those obtained by Tobias (1948) with this tissue. The mean values for the nerve cord tissue were also of the same order as those given by Tobias, while the concentrations in the haemolymph were similar to the values of 164.2 mM./l. for sodium and 7.4 mM./l. for potassium obtained by Asperen & Esch (1956).

Table 1. *Concentration of sodium and potassium in the haemolymph and abdominal nerve cord of adult male cockroaches*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Sodium concentration (mM./l. ± s.D. (n))</th>
<th>Potassium concentration (mM./l. ± s.D. (n))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolymph</td>
<td>157.4 ± 28.0 (15)</td>
<td>12.3 ± 2.0 (15)</td>
</tr>
<tr>
<td>Nerve cord</td>
<td>103.2 ± 23.7 (14)</td>
<td>180.2 ± 26.1 (14)</td>
</tr>
</tbody>
</table>

The freezing-point depression of the haemolymph of adult males was also measured, the data being summarized in Table 2.

To inject the $^{24}$Na and the $^{42}$K into the haemolymph an experimental solution was devised in which the concentrations of the various salts and organic substances

Table 2. *The freezing-point depression of haemolymph from adult male cockroaches*

<table>
<thead>
<tr>
<th>Serial</th>
<th>$\Delta^\circ$ C.</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.960</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.890</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.880</td>
<td>0.897</td>
</tr>
<tr>
<td>4</td>
<td>0.895</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.860</td>
<td></td>
</tr>
</tbody>
</table>
Fluxes in the abdominal nerve cord of the cockroach

approximated to those of the haemolymph. The substances incorporated in this solution are tabulated in Table 3. The sodium and potassium concentrations used in this solution were the same as those given in Table 1, the concentrations of the other ions being based on the values given by Asperen & Esch (1956) for cockroach haemolymph. The concentrations of trehalose and glucose were taken from the data of Treherne (1960). The total osmotic pressure of the solution was adjusted by the addition of the three amino-compounds, which were found by two-dimensional paper chromatography to be present in relatively large amounts in the haemolymph of this insect. The freezing-point depression of this fluid was 0.89°C, which approximated to the value of 0.897°C for the haemolymph.

Table 3. The composition of the experimental solution used in this investigation

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (mm./l.)</th>
<th>Substance</th>
<th>Concentration (mm./l.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>157.0</td>
<td>Trehalose</td>
<td>36.9</td>
</tr>
<tr>
<td>K</td>
<td>12.3</td>
<td>Glucose</td>
<td>2.2</td>
</tr>
<tr>
<td>Ca</td>
<td>4.5</td>
<td>Glycine</td>
<td>30.0</td>
</tr>
<tr>
<td>Mg</td>
<td>4.9</td>
<td>Glutamic acid</td>
<td>35.0</td>
</tr>
<tr>
<td>Cl</td>
<td>184.1</td>
<td>Glutamine</td>
<td>30.0</td>
</tr>
<tr>
<td>H₂PO₄</td>
<td>0.1</td>
<td>Δ</td>
<td>0.89°C</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. The increase in specific activity of potassium in the nerve cord, relative to that in the haemolymph, following the injection of ⁴¹K into the haemolymph.

The penetration of injected ⁴¹K into the abdominal nerve cord from the haemolymph is illustrated in Fig. 1, in which the specific activity in the nerve cord relative to that in the haemolymph is plotted with respect to time. These results showed that the time for half-exchange of the nerve cord potassium with the ions in the haemolymph was approximately 24 min.

To interpret the results illustrated in Fig. 1 it is necessary to calculate the rate of influx of potassium ions into the nerve cord. Such a calculation in this in vivo system would involve a knowledge of the changes in the specific activity of the haemolymph
potassium following injection. This decline in specific activity, which would tend to be minimized by the large volume of fluid injected, was unfortunately obscured by the large individual variation. Thus, following Shaw (1958), these influxes have been very approximately calculated using equations describing the exchange of ions between isolated living cells and the surrounding media (cf. Harris & Burn, 1949):

\[ m_t = -\frac{1}{t} c_t \ln \left( 1 - \frac{a_{in}}{a_{out}} \right), \]

where \( m_t \) = inward ionic flux, \( t = \) time, \( c_t = \) concentration of ion in nerve cord, \( a_{in} \) and \( a_{out} = \) specific activities in the nerve cord and haemolymph respectively.

Fig. 2 illustrates the semi-logarithmic plot of \( c_t \ln (1 - [a_{in}/a_{out}]) \) with respect to time. The line drawn through the points is the average of the angles formed by the lines joining the individual readings to the origin (Treherne, 1954). From the slope of the line shown in Fig. 2 it can be calculated, using equation (1), that the influx of potassium ions was equivalent to 312 mM./l. nerve cord water/hr.

![Fig. 2. The calculated influx of potassium ions into the nerve cord derived from the data in Fig. 1.](image)

The entry of the injected \(^{24}\)Na from the haemolymph is represented in Fig. 3 in which the ratio of the specific activities in the nerve cord and the haemolymph are plotted with respect to time. The data plotted as \( c_t \ln (1 - [a_{in}/a_{out}]) \) against time is illustrated in Fig. 4. From the slope of the calculated line drawn through the points it is possible to estimate that the influx of sodium ions was approximately 320·0 mM./l. nerve cord water/hr.

The sodium and potassium contents of the haemolymph and the nerve cord from the individuals used in these experiments are summarized in Table 4.

Because of the great differences in the concentrations of sodium and potassium in the haemolymph it is necessary to convert the calculated influxes for these ions to...
Fluxes in the abdominal nerve cord of the cockroach

Fig. 3. The increase in specific activity of sodium in the abdominal nerve cord, relative to that in the haemolymph, following the injection of $^{24}$Na into the haemolymph.

Fig. 4. The influx of sodium ions calculated from the data illustrated in Fig. 3.

Table 4. Concentration of sodium and potassium ions in the haemolymph and abdominal nerve cord of individuals used in $^{24}$Na and $^{40}$K uptake experiments

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Sodium concentration (mM/l $\pm$ S.D. ($n$))</th>
<th>Potassium concentration (mM/l $\pm$ S.D. ($n$))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolymph</td>
<td>$132.4 \pm 20.7$ (26)</td>
<td>$9.2 \pm 3.2$ (24)</td>
</tr>
<tr>
<td>Nerve cord</td>
<td>$98.3 \pm 19.2$ (26)</td>
<td>$182.3 \pm 22.8$ (24)</td>
</tr>
</tbody>
</table>
transfer constants in order to compare their relative rates of penetration. A transfer constant can be related to the influx by the equation

\[ k_{in} = \frac{m_i}{c_0}, \]

where \( c_0 \) is the concentration of the ion in the haemolymph and \( k_{in} \) is the transfer constant in the direction in \( \rightarrow \) out. \( k_{in} \) is thus the flux per unit outside concentration, having the units \( 1/time (\text{hr.}^{-1}) \). This constant, \( k_{in} \), is not comparable with the \( k \) of Keynes & Lewis (1951), which is equivalent in this case to \( k_{out} \). The values for \( k_{in} \) derived from the calculated influxes then become:

\[
\begin{align*}
\text{sodium } k_{in} &= \frac{320.0}{132.4} = 2.41 \text{ hr.}^{-1}, \\
\text{potassium } k_{in} &= \frac{312.0}{9.2} = 33.9 \text{ hr.}^{-1}.
\end{align*}
\]

To calculate the flux per unit area of nerve cord surface it is necessary to know the volume per unit area of this organ. In order to obtain an approximate idea of this ratio some isolated abdominal nerve cords were placed in liquid paraffin and their dimensions measured with a travelling microscope. The area and volume of the connectives were calculated by considering them as cylindrical structures. The values for the individual ganglia were estimated by considering these structures as oblate or prolate spheroids. By these rather crude methods the surface/volume ratio was estimated to be roughly about \( 6.4 \text{ cm.}^{-1} \). The influx per unit area of nerve cord surface can thus be represented as:

\[
\begin{align*}
\text{sodium } &13.9 \times 10^{-12} \text{ M cm.}^{-2} \text{ sec.}^{-1}, \\
\text{potassium } &13.5 \times 10^{-12} \text{ M cm.}^{-2} \text{ sec.}^{-1}.
\end{align*}
\]

DISCUSSION

The results outlined above indicate that there is a relatively rapid exchange of sodium and potassium ions between the abdominal nerve cord and the haemolymph in this insect. In fact the movements of the labelled potassium ions through the membrane surrounding the central nervous system in this insect were not far removed from those which have been measured in individual cephalopod and crustacean axons. In the nerve cord the estimated influx of potassium ions of roughly \( 13 \times 10^{-12} \text{ M cm.}^{-2} \text{ sec.}^{-1} \) approached the value of \( 16.7 \times 10^{-12} \text{ M cm.}^{-2} \text{ sec.}^{-1} \) obtained for single resting Sepia axons by Keynes (1951). These figures are more or less directly comparable for the outside concentrations of potassium were rather similar in the two cases, being \( 9.2 \text{ mM./l.} \) in the cockroach haemolymph and \( 9.7 \text{ mM./l.} \) in the artificial sea water used by Keynes. The movement of \( ^{24}\text{Na} \) into the cockroach abdominal nerve cord, approximately equivalent to \( 13.9 \times 10^{-12} \text{ M cm.}^{-2} \text{ sec.}^{-1} \), was rather less than in the isolated Sepia axons where the measured influx across the resting membrane was \( 61.0 \times 10^{-12} \text{ M cm.}^{-2} \text{ sec.}^{-1} \). In this case, however, the concentration of \( 45.8 \text{ mM./l.} \) \( \text{Na}^+ \) in the artificial sea water employed in the Sepia preparations exceeded that in the cockroach haemolymph by almost three times; thus it seems that the sodium movements through the perilemma were of a similar order of magnitude to those for the cephalopod axons.
Fluxes in the abdominal nerve cord of the cockroach

Comparison of the transfer constants for the entry of the ions into the nerve cord, where $k_{in}$ for sodium was 2.4 hr.$^{-1}$ and for potassium was 33.9 hr.$^{-1}$, indicate that the movements of the individual potassium ions occurred about fourteen times more rapidly than those of the sodium ions.

The rapidity of these ionic movements through the perilemma are perhaps rather surprising in view of the observations by Hoyle (1953) and Twarog & Roeder (1956) that the membrane surrounding the nervous system apparently serves a protective function enabling the underlying nervous elements to function despite wide variations in the ionic composition of the surrounding media. These authors showed that in Locusta nerve fibres, and in the abdominal nerve cord of Periplaneta, removal of the continuous sheath associated with these structures resulted in a rapid interference with nervous function in the presence of excess potassium ions. The perilemma of the cockroach nerve cord seems to be less efficient in this respect than the nerve sheath demonstrated by Hoyle in the locust, for the Locusta nerve resisted block for as long as 4 hr. in a solution containing 140 mM./l. K$^+$ as compared with the cockroach nerve cord which became blocked in 22–30 min. in 140 mM./l. K$^+$ and in 12–18 min. in 180 mM./l. K$^+$. These experiments seem to have led to the general assumption that the sheath was functioning as a diffusion barrier restricting the entry of ions into the underlying nervous tissues. The possibility certainly exists that the membrane bounding the central nervous system may be impermeable to net ionic movements, the influxes measured in this investigation being largely due to some sort of exchange diffusion mechanism of the type postulated by Ussing (1949). However, Tobias (1948) has shown that the potassium content of the cockroach nerve cord increased rapidly following a rise in the level of the haemolymph potassium, from an average of 27.1 mM./l. to 49.2 mM./l., produced by the ingestion of 1.4 N-KCl. In view of the fairly massive net movements of potassium into the nerve cord demonstrated by Tobias it must be concluded that the sort of impermeability postulated above does not play an important part in this system. The present experiments clearly suggest then that a dynamic steady state rather than a static impermeability must exist across the perilemma in this insect. The important observation by Hoyle that impairment of the tracheal supply reduced the efficiency of the perilemma may be a reflection of this dynamic state, for if the membrane functioned merely as a static diffusion barrier then it might not be expected to be closely linked to the metabolism of the system.

It has been often assumed in the past that the selective permeability of the perilemma was due to the outer fibrous sheath, the neural lamella. It is difficult to visualize how such a membrane could function to maintain a dynamic equilibrium between the haemolymph and the central nervous system. The observations of Twarog & Roeder (1956) who found that silver nitrate penetrated the fibrous layer, and of Wigglesworth (1960) who found that the neural lamella was apparently freely permeable to dye molecules, suggest that it is the cellular perineurium which is effective in regulating the fluxes of ions and molecules across the perilemma.

These experiments showed that the greater part of the sodium and the potassium within the central nervous system exchanged completely and rapidly with the labelled ions in the haemolymph, for in both cases the specific activities within the nerve approached 1.0. Thus there is no evidence in this system for any appreciable amounts
of very slowly exchanging sodium or potassium such as have been postulated to occur in nerve by Rothenberg (1950).

The results outlined here do not, of course, throw much light on the nature of the processes involved in the transfer of ions between the haemolymph and the central nervous system in this insect. It is hoped that in future investigations some of the factors associated with these ionic transfers may be elucidated.

**SUMMARY**

1. The influx of sodium and potassium ions into the central nervous system of *Periplaneta americana* has been studied by measuring the increase in radioactivity within the abdominal nerve cord following the injection of $^{24}$Na and $^{42}$K into the haemolymph.

2. The calculated influx of sodium ions was approximately 320 mM./l. of nerve cord water/hr. and of potassium ions was 312 mM./l. of nerve cord water/hr. These values are very approximately equivalent to an influx per unit area of nerve cord surface of $13.9 \times 10^{-12} \text{ M cm}^{-2} \text{ sec}^{-1}$ for sodium and $13.5 \times 10^{-12} \text{ M cm}^{-2} \text{ sec}^{-1}$ for potassium ions.

3. The relatively rapid influxes of these ions are discussed in relation to the postulated function of the nerve sheath as a diffusion barrier. It is suggested that a dynamic steady state rather than a static impermeability must exist across the sheath surrounding the central nervous system in this insect.

**REFERENCES**


