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THE MOVEMENTS OF SODIUM IONS IN THE ISOLATED ABDOMINAL NERVE CORD OF THE COCKROACH, *PERIPLANETA AMERICANA*

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INTRODUCTION

In an earlier investigation it was demonstrated that the exchange of sodium and potassium ions between the haemolymph and the central nervous system of *Periplaneta* occurred relatively rapidly (Treherne, 1961). On the basis of these results it was concluded that a dynamic steady state rather than a static impermeability must exist across the perilemma in this insect. The experiments described in this paper represent an attempt to throw some further light on the nature of the ionic movements between the haemolymph and the central nervous system in the cockroach.

METHODS

The abdominal nerve cords used in these experiments were loaded with radiosodium by the injection into the haemolymph of 100 μl. of a solution containing ^{24}Na (0·1–0·5 mc./ml.). After 30 min. the abdominal nerve cord was quickly removed from the insect, by dissection from the dorsal surface, and cleaned of adhering tissue by lightly drawing it across a piece of filter paper. In a limited number of experiments the nerve cords were loaded *in vitro* by soaking in an oxygenated solution containing ^{24}Na. The abdominal nerve cord was ligatured at the connective anterior to the first abdominal ganglion and immediately in front of the terminal abdominal ganglion. This preparation was then quickly washed in Ringer solution, tied to a length of glass rod and placed in the apparatus used to measure the sodium efflux of the abdominal nerve cord.

The apparatus used in these experiments consisted of a small Perspex trough, the floor of which was formed by a layer of 0·00025 in. terylene sheet of minimal stopping power to the β and γ radiations emitted by ^{24}Na. Immediately beneath the terylene window was a GM tube (Mullard MX123) which was linked to a scalar unit (Panax 100c). Oxygenated Ringer flowed through the trough at a rate of approximately 50 ml./min. and the decline in radioactivity associated with the nerve cord was followed throughout the experiment. The volume of the fluid contained in the Perspex trough was 0·45 ml. The whole of the apparatus was housed in a lead castle to minimize the effects of extraneous radiations. This apparatus is essentially similar in principle to that used by Hodgkin & Keynes (1955) to study the loss of sodium ions from isolated squid giant axons.

The solution used for loading the nerve cords with ^{24}Na was that devised by Treherne (1961) and is summarized in the first column of Table 1. The relatively high concentrations of trehalose and glutamine used in this solution made it too expensive for use with the large volumes necessary in the efflux experiments. A second solution was, therefore, used in these experiments in which the trehalose was replaced by sucrose and the glutamine by an increase in the concentration of glucose (column 2, Table 1). Sodium-free solutions were prepared by replacing the sodium salts with choline chloride (column 3, Table 1) or by an appropriate concentration of xylose (column 4). In the remaining solution used in these experiments the potassium was replaced by a proportional increase in the NaCl content (column 5).

Table 1

Substance	Normal Ringer (mM./l.)	Efflux Ringer (mM./l.)	Na-free Ringer (mM./l.)	Na-free Ringer (mM./l.)	K-free Ringer (mM./l.)
NaCl	154·8	154·8	—	—	167·1
KCl	12·3	12·3	12·3	12·3	—
$CaCl_2$	4·5	4·5	4·5	4·5	4·5
$MgCl_2$	4·0	4·0	4·0	4·0	4·0
$NaHCO_3$	2·1	2·1	—	—	2·1
NaH_2PO_4	0·1	0·1	—	—	0·1
$KHCO_3$	—	—	2·1	2·1	—
KH_2PO_4	—	—	0·1	0·1	—
Trehalose	36·9	—	—	—	—
Sucrose	—	36·9	36·9	36·9	36·9
Xylose	—	—	—	281·2	—
Glucose	2·2	35·0	35·0	35·0	35·0
Glutamic acid	35·0	35·0	35·0	35·0	35·0
Glutamine	30·0	—	—	—	—
Glycine	30·0	30·0	30·0	30·0	30·0
Choline chloride	—	—	157·0	—	—

RESULTS

The decline in radioactivity of a ^{24}Na-loaded isolated abdominal nerve cord maintained in flowing Ringer solution is illustrated in Fig. 1. In all of these experiments there was an initial approximately exponential decline in radioactivity which eventually appeared to give way to a second slower phase which also approximated to an exponential curve. Unfortunately the second phase occurred in a region of very low activity and could, for example, have resulted from some errors in the estimation of the background count, although the fact that it was present in all of the experiments would make this seem rather unlikely.

It seemed possible that some of the loss of ^{24}Na from the isolated nerve cord could have occurred from the cut ends of the segmental nerves. This possibility was eliminated in some experiments by following the decline in radioactivity associated with single ligatured abdominal connectives. The connectives used were those from between the fourth and fifth abdominal ganglia. The efflux of ^{24}Na from this preparation appeared to be essentially similar to that from the whole abdominal nerve cord (Fig. 2).

When the nerve cord was loaded with ^{24}Na *in vitro*, by soaking it in radioactive Ringer solution, the initial rapid phase was significantly reduced. Fig. 3 illustrates

the rate of loss of radiosodium from a nerve cord loaded in isolation for 10 min. where the apparent onset of the slow phase occurred after less than 5 min. and at a relatively high level of radioactivity within the nerve cord. In this case then the slow exponential phase cannot be attributed to errors in the estimation of the background activity.

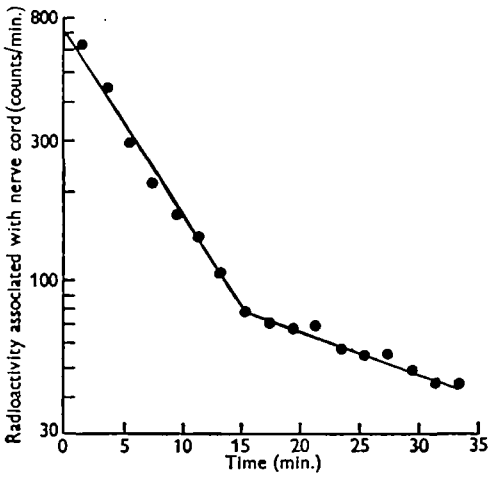

Fig. 1

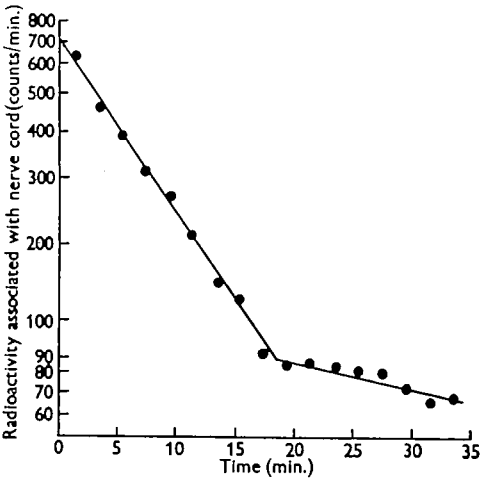

Fig. 2

Fig. 1. The efflux of ^{24}Na from an isolated abdominal nerve cord.

Fig. 2. The efflux of ^{24}Na from an isolated abdominal connective.

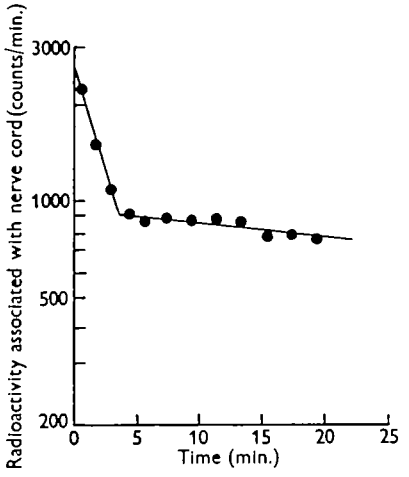

Fig. 3

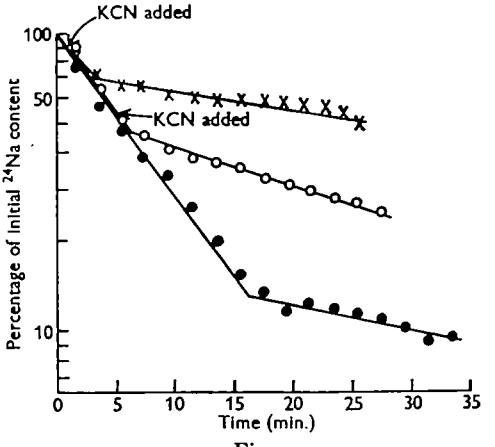

Fig. 4

Fig. 3. The efflux of ^{24}Na from an isolated nerve cord which was loaded *in vitro* for 10 min.

Fig. 4. The effect of 10·0 mM./l. KCN on sodium efflux.

The action of potassium cyanide on the rate of loss of sodium ions is shown in Fig. 4. In these experiments the normal solution was changed, after varying periods, to one containing in addition 10·0 mM./l. KCN. The addition of the poison resulted after a short delay period in a slowing down of the efflux of sodium ions.

The action of 2:4-dinitrophenol at a concentration of 0·5 mM./l. was essentially similar to that of cyanide and resulted in every case in a clear-cut reduction in the rate of loss of sodium ions from the abdominal nerve cord (Fig. 5).

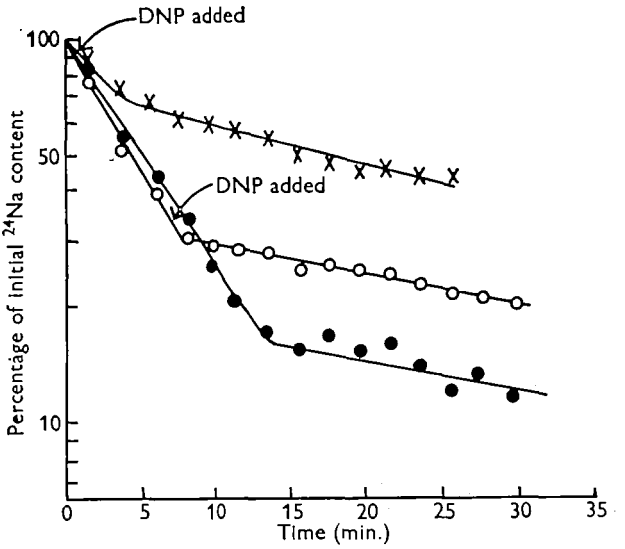

Fig. 5. The effect of 0·5 mM./l. 2:4 dinitrophenol on sodium efflux.

In the next group of experiments the effect of the external sodium concentration on the rate of loss of radiosodium ions from the abdominal nerve cord was investigated. Fig. 6 shows the decline in radioactivity of an abdominal nerve cord in which a sodium-free solution was substituted for the normal Ringer after 8 min. This solution, in which the sodium was replaced by choline, produced no marked effect on the rate of loss of sodium ions from the nerve cord. Similarly in experiments in which the sodium was replaced by an appropriate concentration of xylose there was very little effect on the initial exponential efflux from the nerve cord (Fig. 7).

The rate of loss of sodium from the nerve cord in the potassium-free solution is illustrated in Fig. 8. In this case there was slowing down in the rate of loss of sodium ions in the potassium-free medium. On return to the normal solution the efflux appeared to continue at the rapid rate before the onset of the final slow phase of sodium loss from this system. There was some variation in extent of the effect of the potassium-free solution on sodium loss. This variation can be seen in the results summarized in Table 2.

DISCUSSION

The initial exponential efflux of radiosodium from the abdominal nerve cord has been shown to be significantly reduced by cyanide and dinitrophenol which clearly suggests that the exit of sodium ions measured in these experiments is not a passive process, but is linked in some way with the metabolism of the system. This inhibitory effect is consistent with the results which have been obtained with several other cells and tissues including the squid axon (Hodgkin & Keynes, 1955). Dinitrophenol, at the order of concentration used in these experiments, is generally held to interfere

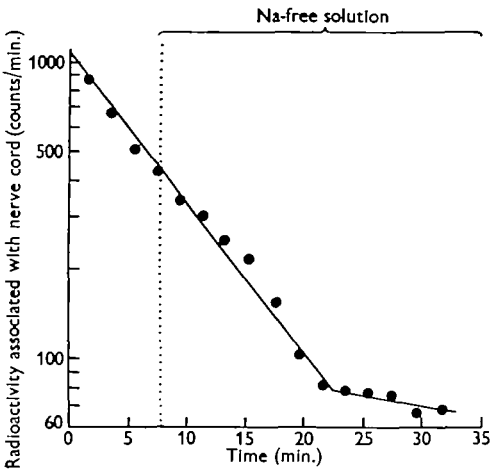

Fig. 6

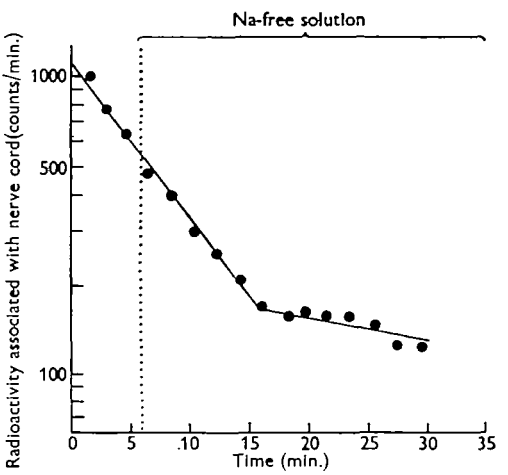

Fig. 7

Fig. 6. The efflux of sodium in a normal solution and in one in which the sodium was replaced by choline.

Fig. 7. The efflux of ^{24}Na in a normal solution and in one in which the sodium was replaced by xylose.

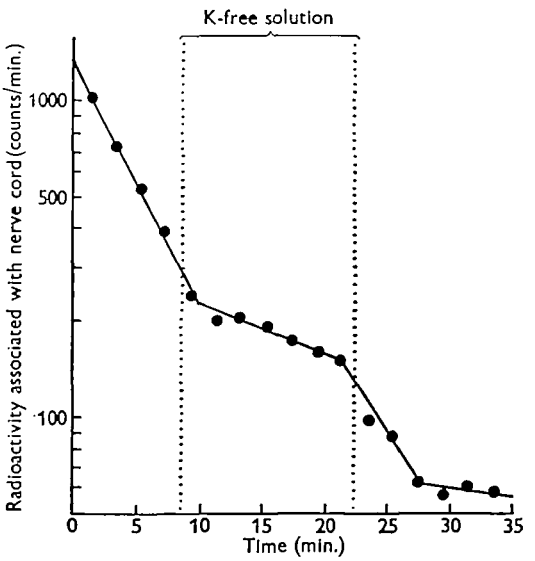

Fig. 8. The effect of potassium-free solution on ^{24}Na efflux from the isolated abdominal nerve cord.

with oxidative phosphorylation and in fact a dependence of sodium extrusion on the presence of ATP has been recently demonstrated in the squid axon (Caldwell, Hodgkin, Keynes & Shaw, 1960).

The extrusion of sodium from the isolated nerve cord appeared to approximate to a two-stage process—an initial rapid exponential phase eventually giving way to a slower component. The second phase was unfortunately difficult to establish in these experiments as it occurred in a region of very low activity, although its presence can perhaps

Table 2. *The effect of potassium-free solution on the rate of sodium efflux ($t_{0.5}$) from isolated nerve cords*

Serial	$t_{0.5}$ Normal solution (min.)	$t_{0.5}$ K-free solution (min.)
1	5·5	19·4
2	5·2	11·8
3	4·8	11·6
4	5·0	35·0
5	5·4	12·6
6	4·9	12·2
7	6·0	25·2

be inferred from the observations on nerve cords loaded *in vitro* in which a slow phase was demonstrated at a high level of radioactivity. There is, of course, no *a priori* reason for assuming that sodium efflux from this organ should be a simple exponential process. It might be imagined, for example, that such an effect could result from the presence of rapid and slowly exchanging fractions within the nerve cord, although it might not perhaps be expected that the phase change would occur so abruptly. It is, however, very difficult to visualize how in such a system a short *in vitro* loading with ^{24}Na could result in an apparent increase in the slowly exchanging sodium fraction. The similarity of the second phase of sodium efflux to that obtained in the poisoned preparations (Figs. 4, 5) might suggest that this slow phase does in fact represent some sort of breakdown of the normal extrusion mechanism in the isolated nerve cord. This evidence is of course entirely circumstantial, but it is perhaps significant to recall the observation of Hoyle (1953, p. 123) that the nerve cord of *Locusta* only showed normal electrical activity for relatively short periods when isolated and separated from its tracheal supply.

The rate of loss of ^{24}Na from the isolated nerve cord was not appreciably reduced when the external sodium was replaced by choline or xylose. Thus, as was suggested in an earlier paper (Treherne, 1961), the extrusion of sodium ions from the central nervous system of this insect does not appear to be part of any 'exchange diffusion' mechanism. The fact that the rate of loss of sodium was, however, reduced in the potassium-free solution demonstrates a close relation between sodium efflux and potassium influx. Such a coupling of sodium and potassium movements has been demonstrated in several cells and tissues (cf. Hodgkin, 1958) and has led to the hypothesis that it might be the result of a mechanism by which one sodium ion is extruded for each potassium ion absorbed (Harris, 1954; Hodgkin & Keynes, 1954). In the present experiments the rate of sodium efflux in the potassium-free solution did not fall to the same extent as that in the presence of the metabolic inhibitors. In this case, as with the *Sepia* axon (Hodgkin, 1958), it is very difficult to be sure that the concentration of potassium ions immediately surrounding the nerve cord surface had not been raised by a leakage of potassium which might allow some limited coupled exchanges of sodium and potassium to continue. It is, therefore, not possible to postulate whether the coupling between sodium efflux and potassium influx is a rigid a partial one.

It is now relevant to consider the significance of these observations on sodium efflux in relation to some previous conclusions on the nature of the permeability processes associated with the continuous fibrous and cellular membrane, the perilemma, surrounding the central nervous system in this insect. The work of Twarog & Roeder (1956), following that of Hoyle (1953) on peripheral nerve, showed that in solutions of high potassium concentration the blocking-time was reduced when the nerve cord was partially desheathed. On the basis of these observations it was suggested that the perilemma functioned as a diffusion barrier restricting the movements of sodium and potassium ions and acetylcholine molecules between the haemolymph and the central nervous system. Some more recent work has, however, demonstrated rapid influxes of sugar molecules and of potassium and sodium ions into the abdominal nerve cord of *Periplaneta* (Treherne, 1960, 1961). It was concluded from these results that in fact a dynamic steady state rather than a static impermeability must exist across the perilemma in this insect (Treherne, 1961). This present investigation has shown in addition that this steady state is at least partially effected by a metabolically maintained linked-sodium pump. It seems reasonable to assume that these exchanges between the haemolymph and the central nervous system are regulated by the perineurium or some deeper cellular layer, the overlying fibrous neural lamella being probably freely permeable to ions and molecules (Wigglesworth, 1960).

In some future investigations an attempt will be made to identify more precisely the rate-limiting processes involved in the extrusion of sodium ions from the central nervous system in this insect.

SUMMARY

1. The rate of loss of sodium ions from the abdominal nerve cord of *Periplaneta* has been determined by following the decline in radioactivity of ^{24}Na-loaded nerve cords isolated in flowing Ringer solution.

2. In all of the experiments there was an initial rapid exponential decline in radioactivity which eventually gave way to a second slower phase.

3. The initial exponential extrusion of sodium ions was appreciably reduced by the presence of potassium cyanide and 2:4-dinitrophenol.

4. The rate of sodium efflux was not reduced in sodium-free solutions, but was decreased in the absence of external potassium ions.

5. It is concluded that sodium ions are extruded from the nerve cord by a metabolically maintained secretory mechanism which is also associated with the uptake of potassium ions.

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