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THE EFFLUX OF SODIUM IONS FROM THE LAST ABDOMINAL GANGLION OF THE COCKROACH, *PERIPLANETA AMERICANA* L.

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INTRODUCTION

The exchanges of sodium and potassium ions and of sugar molecules between the haemolymph and the central nervous system of the cockroach have been found to occur relatively rapidly (Treherne, 1960, 1961*a*). A recent investigation has also shown that the measured efflux of sodium ions from the whole abdominal nerve cord of the cockroach was substantially reduced by the presence of metabolic inhibitors and the absence of potassium ions in the external solution (Treherne, 1961*b*). These results clearly suggested that the rate-limiting transfer measured in these experiments was, in fact, some sort of linked-sodium pump. In the present experiments on the rate of loss of ^{24}Na from the isolated terminal abdominal ganglion an attempt has been made to extend the earlier observations and, in particular, to investigate the role of the cellular and fibrous nerve sheath, the perilemma, in the extrusion of sodium ions from the central nervous system of this insect.

METHODS

In these experiments the efflux of ^{24}Na from the isolated terminal abdominal ganglion was studied while maintaining the preparation in a stream of oxygenated physiological solution. The ganglion was made radioactive either by the injection of 100 μl. of a solution containing ^{24}Na (0·1–0·5 mc./ml.) into the haemolymph or by the perfusion of the whole abdominal nerve cord for 30 min. with the solution. The perfusion was carried out on decapitated adult male *Periplaneta americana* L. in which the dorsal integument was cut away and the viscera removed to expose the abdominal nerve cord. The tracheal supply of the nerve cord thus remained intact during the period when the body cavity was flooded with the radioactive solution. The whole radioactive nerve cord was then removed and the terminal ganglion isolated by ligatures at the anterior connectives and the cercal nerves. The ligatures were tied with threads pulled from 15 denier nylon stockings. The isolated ganglion was then tied to a small length of glass rod and mounted in the apparatus used to measure sodium efflux. This apparatus, which was used in an earlier investigation (Treherne, 1961*b*), consisted of a small rectangular Perspex chamber (0·45 ml. volume) the floor of which was formed by 0·00025 in. Terylene sheet of minimal stopping power to the radiations emitted by ^{24}Na. Oxygenated physiological saline flowed through the chamber at a rate of approximately 0·85 ml./sec. A Geiger tube (Mullard MX. 123) was mounted

beneath the Perspex chamber and continuous record was kept of the decline in radioactivity associated with the ganglion using a Panax scaler unit (100 c.).

In some experiments the isolated radioactive ganglia were partially desheathed according to the procedure outlined by Twarog & Roeder (1956). The sheath was lifted and torn gently from the entire dorsal surface of the ganglion using finely ground watchmaker's forceps. According to Twarog & Roeder desheathing involves removal of the outer connective tissue layer and the associated cellular perineurium.

The various solutions used in these experiments were similar to those devised for some previous investigations. The radioactive solution used for injection into the haemolymph and for perfusion of the nerve cord was that given by Treherne (1961*a*). The physiological solutions used in the efflux experiments were the same as those devised for the investigation on the whole abdominal nerve cord (Treherne, 1961*b*). These consisted of a normal solution containing the various ions and molecules in proportions approximately similar to those in cockroach haemolymph together with a modified potassium-free solution in which the KCl (12·3 mM./l.) was replaced by an appropriate increase in NaCl (to 167·1 mM/l.).

The sodium concentrations of the terminal abdominal ganglion were measured by means of an EEL flame photometer. For this purpose batches of three ganglia were weighed on a 5·0 mg. torsion balance and ashed on small pieces of platinum foil in a muffle furnace at temperatures of 460–480° C.

RESULTS

The measurements of the sodium content of the terminal abdominal ganglion are tabulated in Table 1. The mean value of 110·5 ± 19·8 mM./l. is of the same order as the sodium concentrations for whole nerve cords given by Tobias (1948) and Treherne (1961*a*).

Table 1. *The sodium concentration* (mM/l. *tissue water*) *of the last abdominal ganglion*

Serial	Na content (mM./l.)	Mean
1	137·0	
2	114·0	
3	126·1	
4	109·6	
5	107·8	110·5 mM./l.
6	124·4	
7	85·1	
8	112·9	
9	78·8	

Fig. 1 shows the rate of disappearance of the radioactive sodium from an isolated terminal abdominal ganglion when washed in flowing inactive solution. The decline in radioactivity associated with the ganglion appeared to follow an approximately exponential fall for as long as the experiment could be continued. There was no evidence in this preparation of a final slow phase of sodium efflux in a region of low radioactivity such as was obtained with the whole nerve cord (Treherne, 1961*b*). The average half time ($t_{0.5}$) for sodium efflux from the ganglion was 5·57 ± 1·01 min.

In the next group of experiments the effect of removing the connective tissue and cellular sheath from the dorsal surface of the ganglion was tested. In these experiments the initial exponential decline in radioactivity was determined. Following this the ganglion was removed from the apparatus and quickly desheathed. The partially desheathed preparation was then returned to the apparatus and the subsequent rate of sodium efflux determined. Fig. 2 shows a typical result obtained with this procedure. Removal of the sheath appeared to have little effect on the rate of loss of sodium ions from the terminal ganglion. The effect of partial desheathing of the ganglion on sodium efflux is summarized in Table 2.

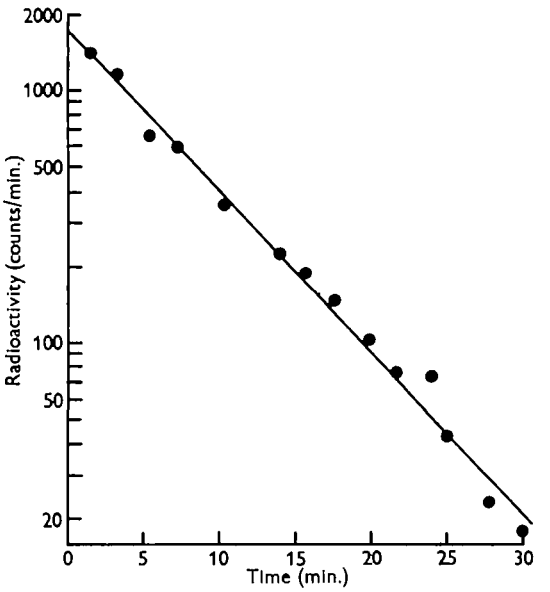

Fig. 1. A semi-logarithmic plot of the decline in radioactivity of a ligatured isolated abdominal ganglion when washed in inactive solution. The ganglion was made radioactive by perfusing the whole abdominal nerve cord with a solution containing ^{24}Na.

The effect of 0·2 mM./l. 2:4-dinitrophenol on the rate of loss of sodium ions from this preparation is illustrated in Fig. 3. Replacement of the normal solution with one containing the poison resulted in a clear-cut slowing down of sodium efflux. Return to the normal solution produced a rapid loss of radioactivity which was followed by an efflux that was slightly slower than the initial rate of sodium loss (Fig. 4). The effect of the presence of 2:4-dinitrophenol on the rate of sodium loss was also tested in the desheathed preparation. In Table 3 the effect of a 0·2 mM./l. solution of the poison on desheathed preparation is compared with that of normal ganglia. These results appeared to show that the poison had less effect on the desheathed as compared with the intact preparation ($P < 0{\cdot}05$).

The effect of transferring the isolated ganglia to a potassium-free solution is illustrated in Fig. 5. In this case the extrusion of ^{24}Na clearly slowed down in the potassium-free solution. On return to the normal solution, containing 12·3 mM./l. K,

there appeared to be a transient rapid efflux which was followed by a rate of sodium loss similar to the initial one. A comparison of the effect of potassium-free solution on normal and desheathed ganglia apparently showed that the effect was less pronounced in the desheathed preparations ($P < 0.05$, Table 4).

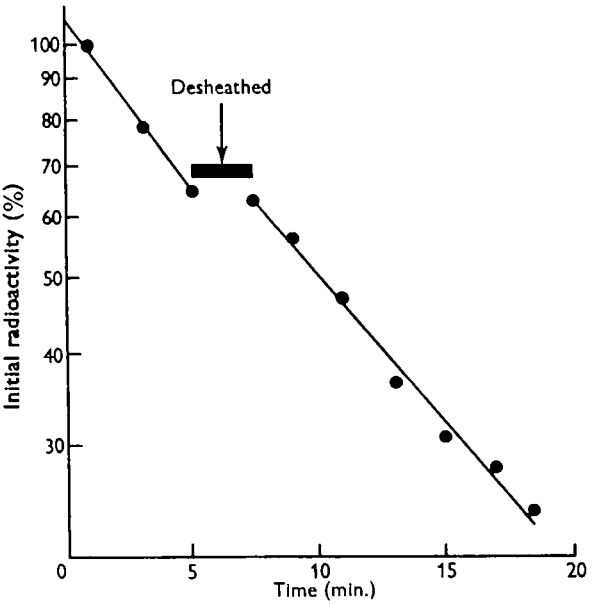

Fig. 2. The effect of removing the connective tissue and cellular sheath from the dorsal surface of the ganglion on the rate of loss of ^{24}Na when washed in inactive solution.

Table 2. *The effect of removal of the perilemma on the half time of sodium loss from the last abdominal ganglion*

Serial	Initial $t_{0.5}$ (min.)	Desheathed ganglion $t_{0.5}$ (min.)
1	5·0	5·3
2	4·7	4·8
3	5·6	5·1
4	6·0	5·9
5	6·8	7·8

DISCUSSION

It is of some interest to compare these results for the isolated abdominal ganglion with those obtained from experiments on the efflux of sodium ions from the whole nerve cord and from isolated connectives (Treherne, 1961*b*). The sodium content of the terminal ganglion was of approximately the same level as that of the whole abdominal nerve cord (Treherne, 1961*a*) so that the rates of efflux, as measured by the decline in radioactivity, are more or less comparable in the different preparations. The values for $t_{0.5}$ appeared to be approximately similar for the connectives (Treherne, 1961*b*) and for the terminal abdominal ganglion used in the present experiments.

These results are perhaps rather unexpected in view of the obvious difference in shape between these two structures. Measurements showed that the surface/volume ratio of the connectives was about 3·5 times greater than that of the relatively massive terminal abdominal ganglion.

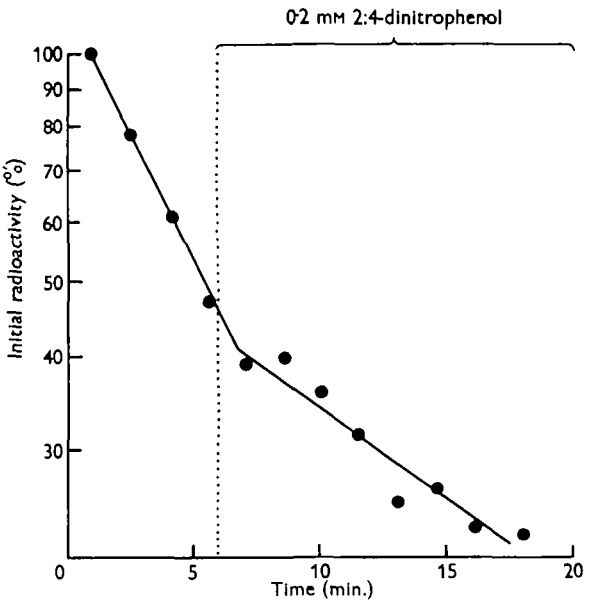

Fig. 3. The effect of 0·2 mM./l. 2:4-dinitrophenol on the escape of ^{24}Na from an isolated terminal abdominal ganglion.

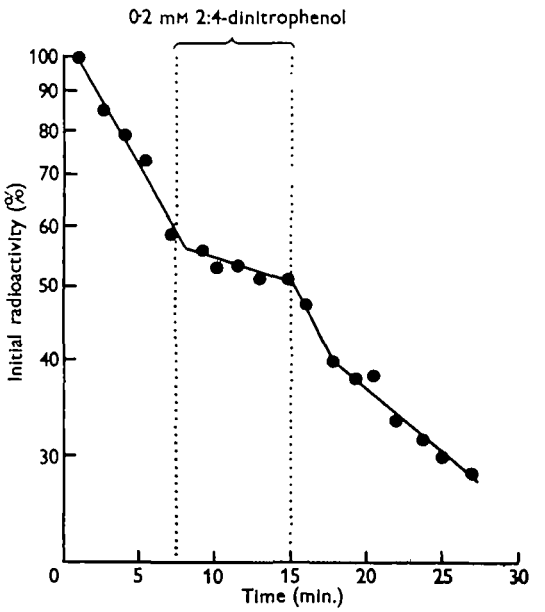

Fig. 4. The effect of 0·2 mM./l. 2:4-dinitrophenol followed by a return to normal physiological solution on the rate of loss of ^{24}Na from the terminal abdominal ganglion.

Table 3. *The effect of* 0·2 mM./l. 2:4-*dinitrophenol on the half time of sodium loss from normal and desheathed ganglia*

Preparation	Serial	Initial $t_{0.5}$ (min.)	Poisoned $t_{0.5}$ (min.)	Ratio $\frac{t_{0.5}\text{ poisoned}}{t_{0.5}\text{ initial}}$	Mean
Normal	1	4·9	17·4	3·7	2·8
	2	11·8	36·0	3·0	
	3	5·5	11·7	2·2	
	4	4·5	11·6	2·6	
Desheathed	5	4·0	8·0	2·0	1·8
	6	3·2	7·0	2·2	
	7	4·6	6·8	1·5	
	8	4·1	6·7	1·6	
	9	4·6	8·5	1·8	

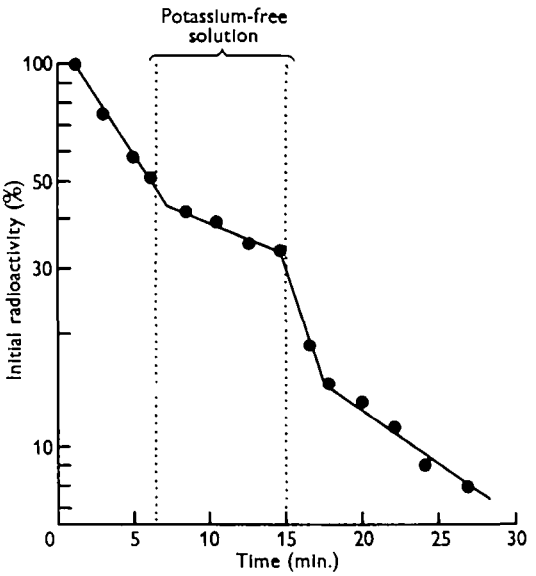

Fig. 5. The effect of potassium-free solution on the rate of loss of ^{24}Na from an isolated abdominal ganglion.

Table 4. *The effect of potassium-free solution on the rate of loss of sodium ions from normal and desheathed ganglia*

Preparation	Serial	Initial $t_{0.5}$ (min.)	K-free $t_{0.5}$ (min.)	Ratio $\frac{t_{0.5}\text{ K-free}}{t_{0.5}\text{ initial}}$	Mean
Normal	1	6·5	13·2	2·0	2·2
	2	7·4	18·0	2·4	
	3	4·9	9·5	1·9	
	4	4·6	12·8	2·8	
	5	4·2	8·3	1·9	
Desheathed	6	4·2	6·3	1·5	1·6
	7	4·4	8·3	1·9	
	8	4·5	6·2	1·4	
	9	5·0	7·5	1·5	
	10	4·0	6·5	1·6	

The point of contrast between the effluxes from the terminal ganglion and from the whole nerve cord used in the previous investigation was the apparent absence, in the case of the isolated ganglion, of a final slow phase of sodium loss in a region of low radioactivity. In the previous study (Treherne, 1961*b*) this phase was tentatively identified with the breakdown of the normal sodium extrusion mechanism in the isolated nerve cord when separated from its tracheal supply. Thus according to this hypothesis it could be postulated that in the present experiments the isolation of the ganglion resulted in a less serious interference with the normal metabolism so that the breakdown of sodium extrusion did not occur until later at a very low level of activity beyond the limits of this technique.

The present results have shown that, as in the whole abdominal nerve cord (Treherene, 1961*b*), the rate of loss of sodium was apparently an active process which was slowed down by the presence of 2:4-dinitrophenol at relatively low concentration. Similarly the extrusion of sodium in the terminal ganglion was reduced in the potassium-free solution, demonstrating a linkage of potassium influx with sodium efflux.

The rate of efflux of sodium ions from the terminal abdominal ganglion was not significantly affected by the removal of about 50% of the connective tissue and cellular sheath. On the basis of these results it must be concluded, therefore, that the rate-limiting process in the efflux of sodium measured by this technique was not the transfer of ions across the cellular perineurium. In addition it follows from this that the diffusion of sodium ions through the connective tissue sheath must also have occurred relatively rapidly, a result which had been previously predicted (Treherne, 1961*a*; Wigglesworth, 1960). The rate-limiting process measured in these experiments must, therefore, be associated with some components of the central nervous system lying at a deeper level than the perineurium. Perhaps the most obvious possibility is that the efflux of ^{24}Na measured in these experiments was, in fact, the result of the transfer of sodium ions across the cell membranes of the underlying tissues. In this case the similarity of the $t_{0.5}$ between the connectives and the terminal ganglion becomes explicable, for under these circumstances the efflux might be expected to be independent of the surface/volume ratio of the whole organ.

The results described above do not, of course, give any definite information about the nature of the processes involved in the passage of ions across the perineurium. However, the fact that the presence of dinitrophenol and potassium-free solution appeared to have slightly less effect on sodium efflux in the desheathed preparations might suggest that this layer of cells perhaps plays more than a passive role in the ionic regulation of the central nervous system of this insect.

The addition of poison to, or the omission of potassium ions from, the external solution has been shown to produce a fairly rapid slowing down of sodium extrusion from the abdominal nerve cord. The fact that the rate-limiting process is not, apparently, the penetration of the superficial perilemma implies that these changes in the chemical composition of the bathing solution are quickly transmitted to the deeper layers of the central nervous system. This conclusion is perhaps rather unexpected in view of the appreciable delay in the breakdown of normal electrical activity obtained when the insect nervous system was exposed to solutions of high potassium concentration (Hoyle, 1953; Twarog & Roeder, 1956).

In some previously published accounts on the entry of ^{42}K and ^{24}Na into the intact abdominal nerve cord of *Periplaneta* (Treherne, 1961*a*, *c*) an attempt was made to calculate the fluxes of these ions between the haemolymph and the central nervous system. These ionic movements were calculated with the conventional equations used to describe fluxes in cells and tissues. This procedure involved the assumption that the rate-limiting process was the transfer across the superficial boundary and that the movements within the underlying layers occurred rapidly so that the labelled ions were effectively well mixed. The present results have shown that these assumptions represented an oversimplification and consequently the calculated values have little significance. It is hoped that in a future investigation the fluxes taking place between the central nervous system and the haemolymph can be calculated for this more complex system.

SUMMARY

1. The rate of loss of ^{24}Na from the terminal abdominal ganglion of *Periplaneta americana* L. has been studied by measuring the decline in radioactivity associated with an isolated preparation maintained in flowing physiological solution.

2. The rate of sodium efflux was substantially reduced in the presence of 0·2 mM./l. dinitrophenol and in potassium-free solution.

3. The extrusion of ^{24}Na was not significantly affected by the removal of the fibrous and cellular sheath surrounding the ganglion. The rate-limiting process in the efflux of sodium measured in the experiments was not, therefore, the transfer of ions across the nerve sheath, but an extrusion from tissues lying at a deeper level in the central nervous system.

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