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THE KINETICS OF SODIUM TRANSFER IN THE CENTRAL NERVOUS SYSTEM OF THE COCKROACH, *PERIPLANETA AMERICANA* L.

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INTRODUCTION

Some previous investigations have shown that the exchanges of sodium and potassium ions between the haemolymph and the cockroach central nervous system occurred relatively rapidly (Treherne, 1961*a*) and appeared to be effected by a mechanism involving an active extrusion of sodium ions (Treherne, 1961*b*). More recently it has also been shown that the measured efflux of sodium ions was not significantly affected by the removal of substantial portions of the cellular and fibrous nerve sheath (Treherne, 1961*c*). It was concluded from this that the rate-limiting factor measured in these experiments was not the transfer of ions across the perilemma but the extrusion of sodium from the underlying tissues of the central nervous system. Thus any rate-limiting movements of ions across the perilemma occurred too rapidly to be measured by the techniques used in the previous investigations. In the present experiments, therefore, an attempt has been made to measure the rapid component of ^{24}Na exchange by determining the rate of loss of radioactivity obtained on washing isolated nerve cords and single connectives and ganglia for relatively short periods in successive volumes of physiological solution.

METHODS

The experiments described in this paper were carried out using the abdominal nerve cords of adult male *Periplanata americana* L. In these experiments the nerve cords were made radioactive by soaking them for varying periods in a solution containing ^{24}Na (0·1–0·5 mc./ml.). With short loading periods (20 sec.–5·0 min.) the isolated ligatured nerve cords were soaked in the oxygenated physiological solution; for longer loading periods (5–20 min.) the nerve cords of decapitated individuals were perfused with the radioactive solution as described in a previous paper (Treherne, 1961*c*). The ligatures were tied with threads pulled from 15 denier nylon stockings. The composition of the radioactive solution used was that given by Treherne (1961*a*). On removal from the radioactive solution the ligatured nerve cords were carefully blotted and then washed for varying periods in successive 0·2 ml. amounts of inactive solution of the same composition. The amount of ^{24}Na remaining in the nerve cord at varying times was determined from the measured radioactivity of the washings. The radioactivity measurements were made with a Mullard MX 123 G.M. tube linked to a 100 c. Panax scaler.

Some preliminary measurements were made to estimate the extent of any 'inulin space' in the central nervous system. This was done by soaking ligatured isolated nerve cords for 1 hr. in a 3·0% solution of ^{14}C-labelled inulin (3·0 mc./g.) made up in physiological solution. The nerve cords were then washed for 25 sec. and the ^{14}C-inulin was extracted by soaking them for 24 hr. in the physiological solution. The washing time of 25 sec. used was found to be the minimum period necessary to remove 97% of the radioactivity from the surface of a nerve cord exposed to ^{14}C-inulin for 1 sec. These values are thus likely to be minimum estimates of the 'inulin space' of this organ for some radioactivity must have leaked from within the nerve cord during the washing procedure. In a limited number of cases the rate of loss of ^{14}C-labelled inulin was determined by washing the ligatured isolated nerve cords in successive volumes of the physiological solution as for the ^{24}Na efflux experiments.

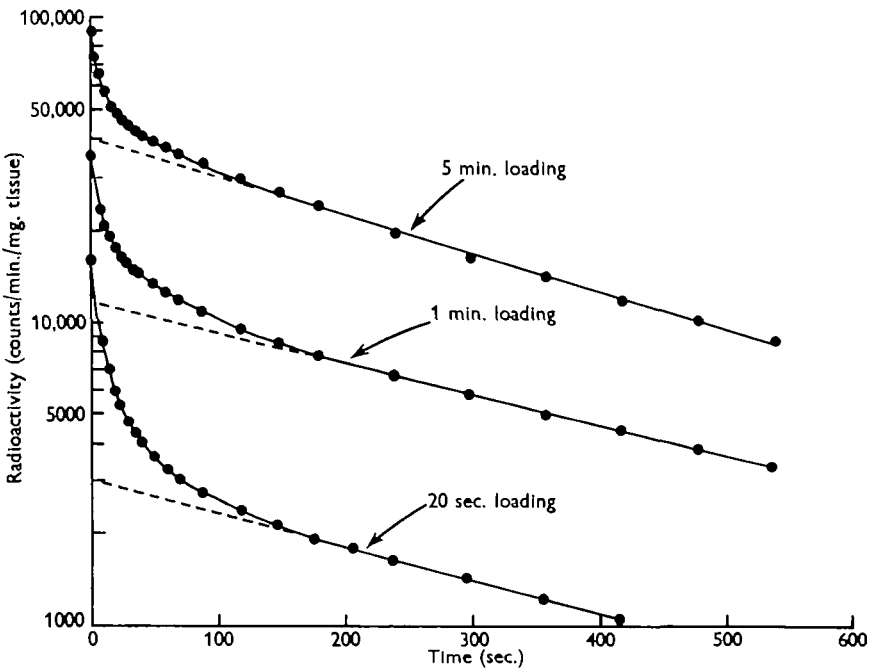

Fig. 1. A semi-logarithmic plot of the decline in radioactivity of abdominal nerve cords, loaded with ^{24}Na for varying periods, when washed in inactive solution.

RESULTS

The results illustrated in Fig. 1 show the decline in radioactivity of some isolated abdominal nerve cords, previously soaked in the solution containing ^{24}Na, when maintained in an inactive solution of the same composition. In all cases semi-logarithmic plots of the results for varying loading times appeared to follow a complex course initially, eventually assuming an exponential form after a period of between 160–200 sec.

It was found possible to separate a fast component from the curves for the loss of ^{24}Na from the nerve cords by subtraction from the initial values lying above the lin

extrapolated to zero time. The separation of an efflux curve into fast and slow components with data plotted semi-logarithmically with respect to time is shown in Fig. 2. The fast component illustrated in Fig. 2 was complex initially, but assumed after a few seconds a simple exponential form with a half-time ($t_{0.5}$) of approximately 33·0 sec. The half-time for the slow component was, in this case, 260 sec.

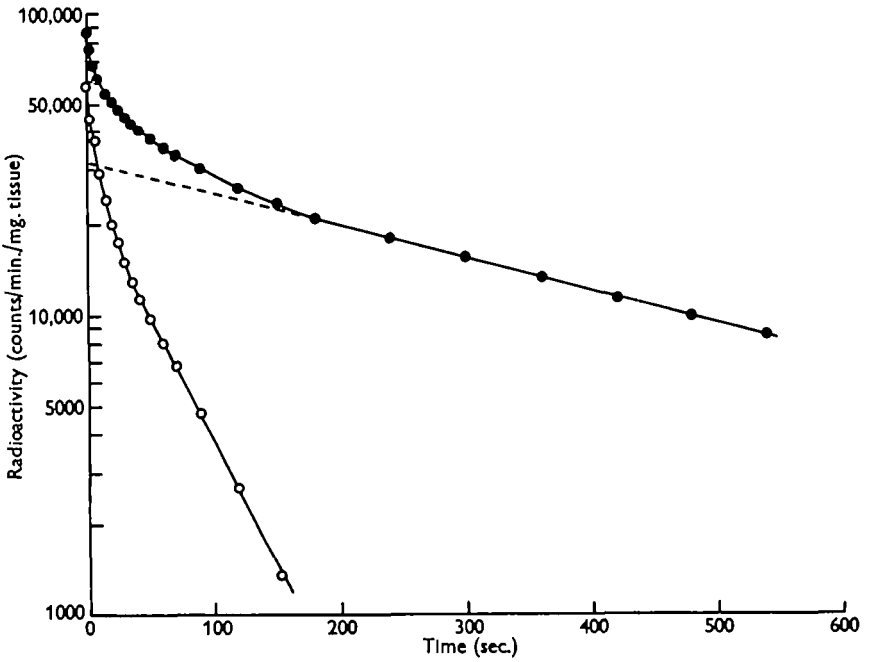

Fig. 2. The loss of ^{24}Na from a nerve cord, loaded for 10 min., when washed in inactive physiological solution (closed circles). The fast component of the main curve (open circles) was obtained by subtraction from the straight line extrapolated to zero time.

The escape of ^{24}Na from the isolated abdominal nerve cords was also measured in the presence of 0·5 mM./l. 2:4-dinitrophenol. The poison was added to the physiological solution during the initial loading period with the ^{24}Na and was present at the same concentration in the inactive solution during the subsequent efflux experiments. Previous results (Treherne, 1961*b*) have shown that there was a slight delay period of a few minutes before the poison affected the rate of extrusion of sodium from the nerve cords. In the present experiments, therefore, the nerve cords which were loaded with ^{24}Na for only short periods (less than 5 min.) were pretreated with 0·5 mM./l. dinitrophenol to maintain a constant exposure to the poison of 5 min. before the efflux experiments were commenced. Fig. 3 shows the escape of ^{24}Na from a poisoned preparation loaded with ^{24}Na for 10 min. In this experiment the fast component was not abolished by the presence of the poison, in fact $t_{0.5}$ in this case was 33·0 sec., which was the same as that for the normal preparation illustrated in Fig. 2. In this particular experiment the slow component for ^{24}Na efflux was, however, much reduced as compared with the normal preparation. The effects of 0·5 mM./l. 2:4-dinitrophenol on the escape of ^{24}Na from the isolated nerve cords are summarized in Table 1. The results

clearly indicate that the presence of the poison affected the slow phase of sodium loss but not the initial fast component.

The total activity of the ^{24}Na in the slowly exchanging fraction was estimated by extrapolation of the slow component to zero time. Fig. 4 illustrates the estimated

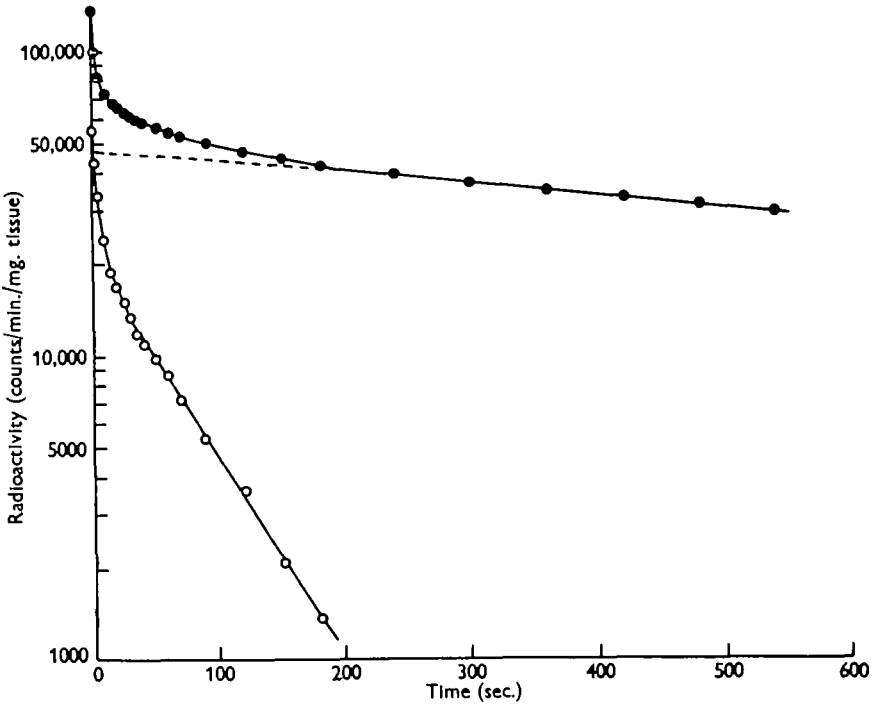

Fig. 3. The escape of ^{24}Na from a nerve cord in inactive solution in the presence of 0·5 mM./l. 2:4-dinitrophenol. The open circles represent the fast component obtained by subtraction from the straight line (closed circles) extrapolated to zero time.

Table 1. *The half-times for the fast and slow components of* ^{24}Na *escape from normal and poisoned abdominal nerve cords*

Experimental conditions	Loading time (sec.)	Fast component $t_{0.5}$ (sec.)	Slow component $t_{0.5}$ (sec.)
Normal	20	22	278
	30	31	264
	60	32	293
	120	23	240
	300	26	240
	600	33	260
	1200	32	360
	Mean	28·4	277·0
0·5 mM./l. 2:4-dinitrophenol	20	31	486
	30	34	498
	60	30	580
	120	36	648
	300	30	546
	600	33	592
	1200	27	800
	Mean	31·5	593·0

adioactivity of the slowly escaping fraction at varying times after exposure to the solution containing ^{24}Na. These data would appear to show that the poison had little effect on the rate of accumulation of the radioactive ions in the slowly exchanging fraction. The results are, however, too few to judge the equilibrium level of radioactivity as between the normal and poisoned preparations.

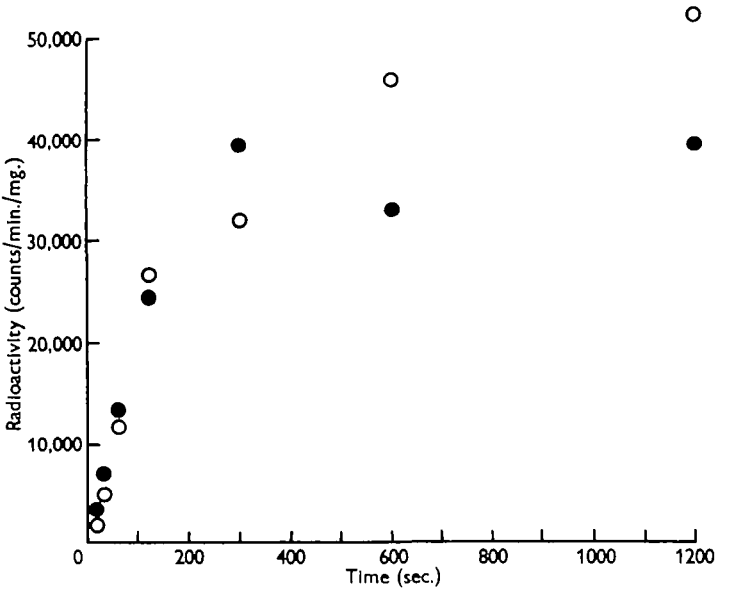

Fig. 4. The estimated activity of the slowly exchanging fraction of the ^{24}Na plotted against time for varying loading periods. The closed circles represent preparations in normal physiological solution; the open circles those in a solution containing 0·5 mM./l. 2:4-dinitrophenol.

The escape of ^{24}Na from isolated ligatured fragments of the central nervous system was studied in some experiments. The loss of radio-sodium from the terminal abdominal ganglion and from the connective between the fourth and fifth abdominal ganglia was found to occur as a two-stage process as for the whole abdominal nerve cord. The results for the escape of ^{24}Na from a single isolated connective are illustrated in Fig. 5. In this case, as for the whole nerve cord, the fast component appeared to be initially complex eventually becoming a simple exponential function. The half-times for ^{24}Na loss were in this experiment: 41·0 sec. for the fast phase and 338·0 sec. for the slow.

The effect of removal of a substantial portion of the perilemma on the rate of loss of sodium was studied in this investigation, by comparison of sodium loss as between an intact isolated terminal abdominal ganglion and one in which the dorsal portion of the cellular and connective tissue sheath was removed with sharpened watchmaker's forceps. Fig. 6 illustrates the fast components for the escape of ^{24}Na from normal and partially desheathed ganglia. The effects of removal on ^{24}Na loss were not obvious, certainly the final exponential phase was not significantly affected by the desheathing. Any effect of this procedure must, therefore, be sought in the initial complex phase which is at the moment very difficult to analyse, especially as the initial portion will also be affected by varying amounts of surface radioactivity.

Preliminary experiments were carried out in an attempt to discover the extent of any extracellular space in the central nervous system of this insect. This was done by the conventional method of measuring the inulin space. ^{14}C-labelled polysaccharide was used. Table 2 shows the inulin space as a percentage of nerve-cord water in washed nerve cords previously soaked for 1 hr. in the radioactive solution.

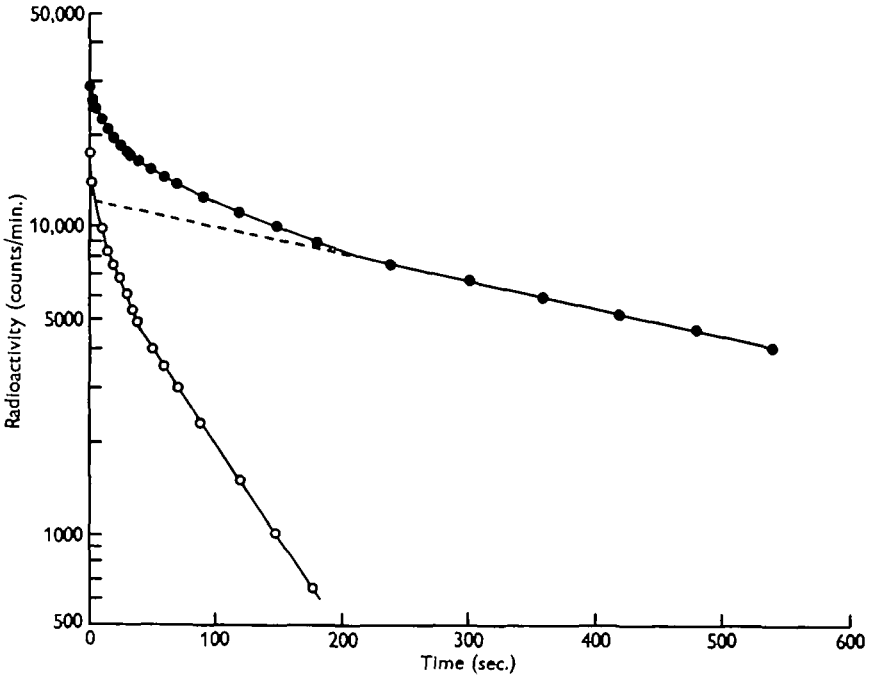

Fig. 5. The fast (open circles) and slow components (closed circles) for the efflux of ^{24}Na from a single connective when washed in inactive solution. The connective was taken from a nerve cord previously soaked for 5 min. in the radioactive solution.

The movements of the ^{14}C-labelled inulin molecules within spaces demonstrated in the previous experiments was studied by determining the rate of loss of radioactivity from the abdominal nerve cord. Fig. 7 shows the measured radioactivity associated with the nerve cord plotted against time when the preparation was washed in inactive physiological solution. These data when plotted logarithmically showed an initial curved portion which eventually gave way to an exponential decline, with a half-time of 214·0 sec.

DISCUSSION

The results outlined above showed that the escape of ^{24}Na from the isolated abdominal nerve cord occurred as a two-stage process—an initial rapid phase, with a half-time of about 28·5 sec., eventually giving way to a slow component, with a half-time of approximately 277·0 sec. The experiments with dinitrophenol clearly indicated that only the slow phase of sodium efflux was reduced by the presence of a metabolic inhibitor, the initial rapid phase being unaffected. The slow phase of sodium loss demonstrated by the present technique thus corresponds both qualitatively and quantitatively with the outward movement of sodium ions from the central

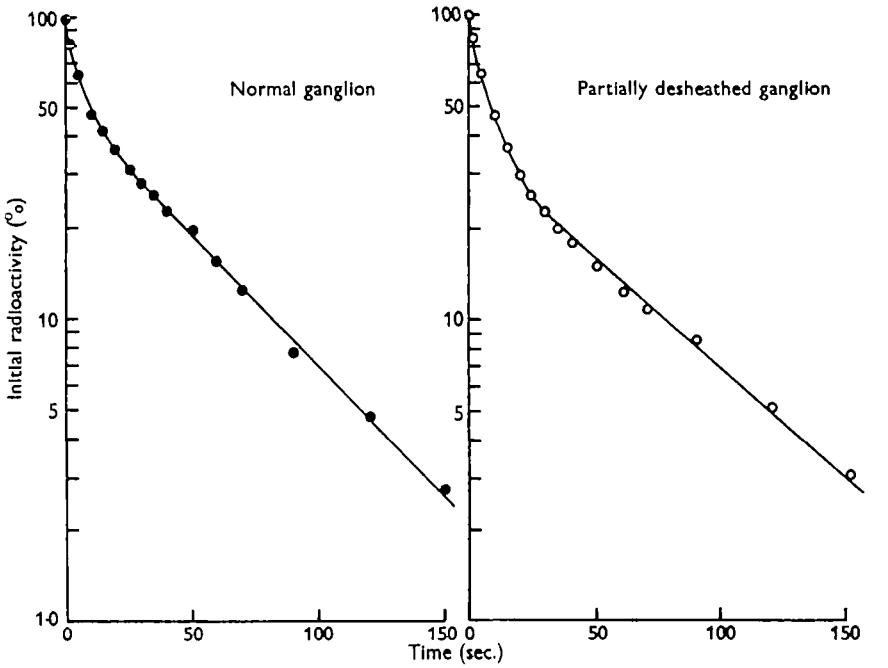

Fig. 6. The fast components obtained in experiments on the escape of ^{24}Na from intact and partially desheathed terminal abdominal ganglia washed in inactive physiological solution.

Table 2. *The inulin space as a percentage of the nerve-cord water, measured in washed preparations*

Serial	Inulin space (%)	Mean
1	9·4	
2	13·1	
3	9·6	
4	13·6	10·1 %
5	6·8	
6	7·9	

nervous system measured in previous investigations (Treherne, 1961*b*; 1961*c*). In the earlier studies in which the radioactive nerve cords were suspended in flowing saline above a GM tube, the technique was not capable of measuring the extremely rapid initial escape of ^{24}Na demonstrated in the present investigation. In these earlier studies then the fast phase of sodium efflux was not recognized.

The question which now has to be considered is the identity of the sodium ions contained in the rapidly exchanging fraction in the central nervous system. The amount of the rapidly exchanging sodium would seem to be too great to attribute this fraction to ^{24}Na associated with the surface of the abdominal nerve cord. For example, in the experiment in which the nerve cord was loaded for 10 min. the radioactivity in the rapidly exchanging fraction would, assuming it to be at the same concentration as in the external solution, account for some 30% of the nerve-cord water. It seems reasonable to suppose then that a substantial proportion of the rapidly exchanging

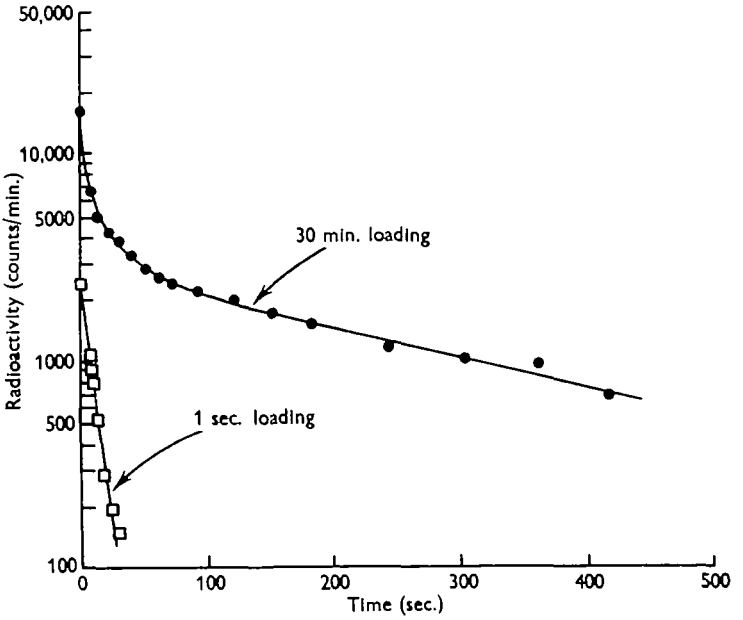

Fig. 7. The escape of ^{14}C-inulin from a nerve cord soaked for 1 sec. and for 30 min. in a 3·0% radioactive solution.

sodium fraction is contained within the central nervous system. This supposition is supported by a consideration of the processes involved in the diffusion of ions and molecules through the spaces in a mass of tissue. According to Hill (1928) diffusion from the extracellular space in a cylindrical muscle will be initially complex but will eventually follow a simple exponential course with a half-time given by

$$t_{0.5} = 0{\cdot}118\, r_0^2/D',$$

where r_0 is the radius of the muscle and D' the diffusion constant in the extracellular spaces. This equation can thus be applied to the data for the loss of ^{24}Na from the single isolated connectives used in the present investigation, in which the average $t_{0.5}$ was 43·0 sec. and r_0 averaged 0·145 mm. The value for D' thus becomes $5{\cdot}77 \times 10^{-7}$ cm.^{2}sec.$^{-1}$, which is approximately one-thirtieth of that for the free diffusion of sodium ions. Such a reduction in the apparent diffusion coefficient could be due, as Harris & Burn (1949) point out, to the increase in the effective path length for ions diffusing between a complex collection of cellular structures. Apparent reductions of between one-eighth and one-sixtyfifth have been noted for the diffusion of sodium ions in the extracellular spaces in cat nerves (Krnjevic, 1955) which is of the same order as for the central nervous system of this insect.

The data for the escape of sodium ions from the central nervous system is thus not inconsistent with the hypothesis that the rapidly exchanging sodium fraction represents the ions contained in extracellular spaces. The experiments with the ^{14}C-inulin demonstrated the existence of a space accessible to the polysaccharide molecules which would occupy at least about 10% of the nerve-cord water. The diffusion of the inulin molecules within the extracellular space can also be compared with that for the loss of ^{24}Na from the abdominal nerve cord. Hill's equation, given above, can b

modified for a complex structure such as the abdominal nerve by representing it thus

$$t_{0.5} = a\frac{I}{D'}$$

where a is a constant. It follows from this that

$$\frac{t_{0.5\ (Na)}}{t_{0.5\ (inulin)}} = \frac{D'_{(inulin)}}{D'_{(Na)}} = \frac{D_{(inulin)}}{D_{(Na)}},$$

where D' is the apparent diffusion constant in the extracellular spaces and D the free diffusion constant. Thus from the experimental data

$$\frac{t_{0.5\ (Na)}}{t_{0.5\ (inulin)}} = \frac{28.5}{214.0} = 0.133$$

and

$$\frac{D_{(inulin)}}{D_{(Na)}} = \frac{0.16 \times 10^{-5}}{1.26 \times 10^{-5}} = 0.127.$$

The two values obtained from these ratios agree reasonably well, which is additional evidence that the fast component in sodium efflux was, in fact, a movement within the extracellular space demonstrated by the ^{14}C-inulin.

The demonstration of a rapid exchange of sodium ions between an extracellular space in the central nervous system and the external solution helps to clarify the results obtained in a previous investigation (Treherne, 1961*c*). In this earlier study it was found that the rate of sodium extrusion from the isolated terminal abdominal ganglion was not significantly affected by the removal of substantial portions of the cellular and fibrous nerve sheath. It has now been shown that the efflux of ^{24}Na measured in the previous experiments corresponded to the slow phase of sodium loss obtained in the present experiments and, in fact, represents an extrusion from the cellular components of the central nervous system. Thus as the relatively slow extrusion takes place into an extracellular space from which ions escape very rapidly it is not surprising that in the previous study removal of the perilemma did not affect the measured loss of sodium ions.

The exchange of sodium ions taking place between the extracellular space and the various cellular components of the cockroach central nervous system appeared to be essentially similar to those taking place in cephalopod giant axons (cf. Hodgkin, 1958). As in the squid axon the accumulation of radioactive sodium within the various cells of the central nervous system of the cockroach appeared to be a passive process, unaffected by the presence of a metabolic inhibitor. The efflux of sodium from the cellular components of the central nervous system was, however, reduced in the presence of metabolic inhibitors as in the cephalopod giant axon. In the absence of external potassium ions (Treherne, 1961*b*, 1961*c*) the extrusion of sodium ions from the cellular components was also reduced indicating that, as in the squid axon, the efflux is effected by some sort of linked sodium pump.

The demonstration of the rapid exchanges of sodium ions between the external solution and an extracellular space in the central nervous system of this insect is perhaps rather unexpected in view of the observations of Hoyle (1953) and of Twarog & Roeder (1956) that the blocking time for insect nerve is dramatically reduced in solutions of high potassium concentration when the perilemma is removed. Twarog & Roeder, for example, demonstrated that in the intact cockroach nerve cord the blocking

time in a solution containing 140 mM./l. potassium was between 22–30 min., as compared to 60–90 sec. in the desheathed preparation. The results have been generally interpreted as indicating that the perilemma functions as a diffusion barrier of some kind. The present experiments have demonstrated, however, an efflux from an extracellular space through the nerve sheath which appears to be a purely passive process and which takes place relatively rapidly ($t_{0.5}$ = 28·5 sec.). It is hoped that some further researches on the cockroach central nervous system may help to throw some light on this apparently paradoxical situation.

SUMMARY

1. The exchange of sodium ions in the cockroach central nervous system has been studied by following the escape of ^{24}Na from isolated abdominal nerve cords, single connectives and ganglia. Particular attention was paid to the initial rapid exchanges of sodium.

2. The escape of sodium ions occurred as a two-stage process, an initial rapid phase eventually giving way to a slower exponential phase of sodium loss. The fast phase of efflux was not affected by the presence of 2:4-dinitrophenol, although this poison significantly reduced the second slow phase of sodium extrusion.

3. The initial fast phase is attributed to a rapid diffusion from an extracellular space, demonstrated by ^{14}C-inulin; the second phase is identified as the slower extrusion from the cellular components of the central nervous system.

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