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THE DISTRIBUTION AND EXCHANGE OF INORGANIC IONS IN THE CENTRAL NERVOUS SYSTEM OF THE STICK INSECT *CARAUSIUS MOROSUS*

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INTRODUCTION

The ionic composition of the haemolymph of some groups of largely phytophagous insects is known to be very different from that of the body fluids of most of the other animals which have been studied (Boné, 1944, 1947; Duchâteau, Florkin & Leclercq, 1953; Sutcliffe, 1963). In these insects the haemolymph is characterized by very low concentrations of sodium ions relative to those of potassium and magnesium. Such an ionic balance presents certain difficulties in the interpretation of the processes involved in nervous transmission in these insects, for in all other animals which have been studied the propagation of nerve impulses has been shown to depend upon high external concentrations of sodium ions (cf. Hodgkin, 1951, 1958; Shanes, 1958*a*, *b*). Thus, for example, a solution approximating to the ionic composition of the haemolymph of *Carausius* resulted in a rapid loss of conduction when applied to the abdominal nerve cord of an insect such as *Periplaneta americana* (Treherne, 1965).

Previous studies on the exchanges of ions and molecules in the insect central nervous system have been carried out using the cockroach, which has a haemolymph containing a normal balance of the common cations (Treherne, 1961*a*, *b*, *c*, *d*, 1962). In these investigations it was shown that the ionic exchanges between the haemolymph and the central nervous tissues occurred relatively rapidly. These exchanges took the form of a rapid movement between the haemolymph and the extracellular fluid, together with slower movements of ions between the extracellular fluid and the cells of the central nervous system. The high level of cations, relative to those of chloride, in the rapidly exchanging extracellular fraction appeared to be governed very largely by a Donnan equilibrium with the haemolymph.

Preliminary experiments on the effects of cations on conduction processes in the nerve cord of *Carausius morosus* showed that, despite their very low concentration in the haemolymph, the presence of sodium ions was essential for the propagation of nerve impulses in this preparation (Treherne, 1965). The presence of magnesium was also essential, for there was a rapid loss of conduction when the nerve cord was bathed in solutions containing reduced concentrations of this cation. Transmission of impulses continued when substantial portions of the nerve sheath were removed from abdominal ganglia, which suggested that the function of the axons in this insect did not depend upon the presence of a peripheral diffusion barrier. These results taken with the

earlier work on the nerve cord of *Periplaneta* throw very little light on the ionic regulation or on the possible mechanisms involved in nervous conduction in the central nervous system of an insect like *Carausius*. The present investigation was therefore undertaken in an attempt to elucidate some of the problems encountered in the neurophysiology of species with such abnormal balances of ions in the haemolymph.

METHODS AND MATERIALS

Many of the techniques used in this investigation were essentially similar to those employed in previous investigations on the central nervous system of *Periplaneta americana* L. (Treherne, 1961*a*, *d*, 1962). Detailed descriptions will, therefore, only be given for techniques which differ from those used in the previous published accounts.

The physiological solution used in this investigation was that devised by Wood (1957) and had the following ionic composition: Na, 15·0 mM./l.; K, 18·0 mM./l.; Ca, 7·5 mM./l.; Mg, 50·0 mM./l.; H_2PO_4, 6·0 mM./l.; HPO_4, 4·5 mM./l.; Cl, 133 mM/l. The pH of this solution was approximately 6·6. The osmotic concentration of the fluid was maintained by the addition of disaccharide, in this case 69·9 g. trehalose/l. in place of the sucrose used by Wood.

The concentrations of sodium, potassium, calcium and magnesium in the nerve cord tissues and in the haemolymph were measured using the Unicam SP 900 flame

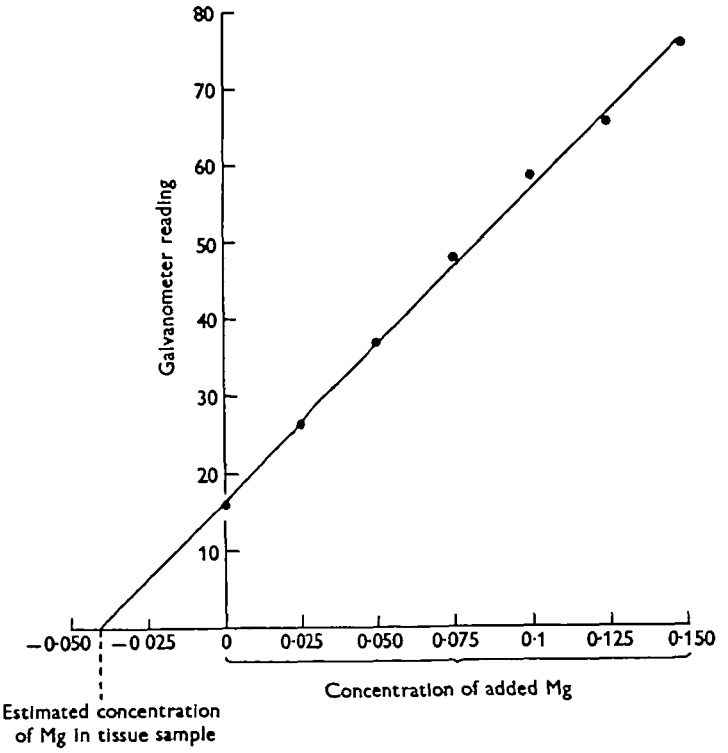

Fig 1. The measured recovery of magnesium ions added to samples of nerve cord tissue.

spectrophotometer. The tissue samples were previously ashed in a muffle furnace on pieces of platinum foil at temperatures of 460–480° C. The direct flame photometric determination of inorganic ions in biological materials is sometimes complicated by the presence of other elements which have various interference effects. A standard check was, therefore, made to ensure that quantitative recoveries of added amounts were obtained for each particular ion (e.g. Fig. 1).

In these experiments the whole ventral nerve cord of *Carausius* was used instead of the abdominal nerve cord preparation employed in the previous investigations with *Periplaneta* (Treherne, 1961*a*, *b*, *d*, 1962). All experiments were carried out at a temperature of about 18·0° C.

RESULTS

(1) *The ionic composition of the haemolymph and nerve cord*

The concentrations of the four main cations in the haemolymph and the tissues of the freshly dissected nerve cord are summarized in Table 1. In each case the water content of the tissues was measured, the individual values being used to calculate the concentrations of the ions in the tissue water.

Table 1. *The ionic composition of the haemolymph and nerve cord of* Carausius morosus

Ion	*n*	Concentration in haemolymph (mM./l. ± S.E.)	Concentration in nerve cord ± S.E. mM./kg. tissue	mM./l. tissue water*
Na	13	20·1 ± 1·6	63·8 ± 8·5	102·4 ± 14·3
K	6	33·7 ± 2·1	313·4 ± 23·4	510·3 ± 31·0
Ca	7	6·4 ± 0·6	30·2 ± 5·9	47·5 ± 7·7
Mg	8	61·8 ± 2·1	22·1 ± 1·5	37·8 ± 2·6

* Average water content: 605·5 ± 17·7 ml./kg. tissue.

The values for the ionic composition of the haemolymph are in approximate agreement with previous measurements which have been made with this insect (Boné, 1944; Duchâteau *et al.* 1953; Ramsay, 1955; Wood, 1957). The value for the sodium concentration is nearer to the figure of 21 mM./l. published by Boné than that of 9·0 mM./l. given by Duchâteau *et al.* The potassium concentration shown here is higher than in the previous investigations. These variations can presumably be attributed to dietary and genetic differences in the various stocks used.

(2) *The measurement of the inulin space in the ventral nerve cord*

These measurements were made using ^{14}C-labelled inulin molecules. The radioactivity associated with the nerve cord tissues was compared as between excised nerve cords exposed to a 1·0% ^{14}C-inulin solution for approximately 1·0 sec. and for 1·0 hr. respectively. These results were also compared with those obtained 3 hr. after the injection of ^{14}C-inulin into the haemolymph (Table 2). It will be seen that in the *in vitro* experiments the radioactivity of nerve cords given the brief exposure to the solution was much less than those soaked for 1 hr. The figure of 12·5 ml./kg. tissue can be used as a measure of the surface contamination by the inulin molecules. The

in vivo experiments showed an apparent inulin space of 104·3 ml./kg. tissue. Applying the figure for the brief exposure as a correction factor for the *in vivo* experiments this would correspond to an estimated extracellular inulin space of 91·8 ml./kg. tissue or 151·6 ml./l. of nerve cord water. It is apparent from the higher figure obtained with the *in vitro* preparation that some increase in extracellular space must have occurred as a result of this treatment.

Table 2. *Uptake of ^{14}C-inulin by nerve cord*

Treatment	Serial	Activity in 1·0 μl. of solution or in haemolymph (counts/min.)	Activity in nerve cord tissue (counts/mg./min.)	Inulin space (ml./kg. tissue)	
Isolated cord immersed 1 0 sec.	1	4690·0	96·3	20·5	12 5 ml /kg.
	2		65 9	14·0	
	3		44·c	9·4	
	4		39 c	8·3	
	5		68·c	14·5	
	6		39·4	8·4	
Isolated cord soaked 1·0 hr.	7	4690·0	603 8	128·7	158·6 ml./kg.
	8		685·0	146·0	
	9		1069·0	227 0	
	10		569·2	121·4	
	11		669·4	142 7	
	12		870·6	185·6	
1·0 % ^{14}C-inulin injected into haemolymph, cord removed after 3 hr.	13	2654·4	347·4	130·9	104·3 ml./kg.
	14	2823 0	230·1	81·5	
	15	2024·1	258 6	127·8	
	16	2973 9	280·0	94·2	
	17	2402·2	250 7	104·4	
	18	2426·4	211·2	87 0	

(3) *Uptake of radioactive sodium by the nerve cord*—in vivo *experiments*

Figure 2 illustrates the changes in specific activity which occur in the tissues of the central nervous system following the injection of 100 μl. of physiological solution containing ^{22}Na. The experimental values approached complete exchange after a period of about 1·0 hr. The changes in the ratio of the specific activities in the nerve cord and the haemolymph occurred at a similar rate to those taking place in the cockroach central nervous system following the injection of ^{24}Na (Treherne, 1961*a*).

(4) *Sodium efflux experiments*

In these experiments 100 μl. of solution containing ^{22}Na was injected into each insect. After 4 hr. the whole ventral nerve cord was removed, carefully blotted on filter-paper and then washed in successive 0·2 ml. volumes of physiological solution. The radioactivities of these washings were measured in a liquid scintillation counter and the amount of radioisotope remaining in the nerve cord at varying times was calculated. Figure 3 is an example of the results obtained in this way. As in previous investigations on the central nervous system of *Periplaneta* this efflux can be represented as a two-stage process, an initial rapid component giving way to a second slow phase.

Table 3 summarizes the results of the experiments on the efflux of ^{22}Na from the nerve cord on washing in inactive physiological solution. It will be seen that approximately a third of the exchangeable sodium was contained in the rapidly exchanging fraction. This can be calculated to be equivalent to 19·5 mM./kg. tissue. These results

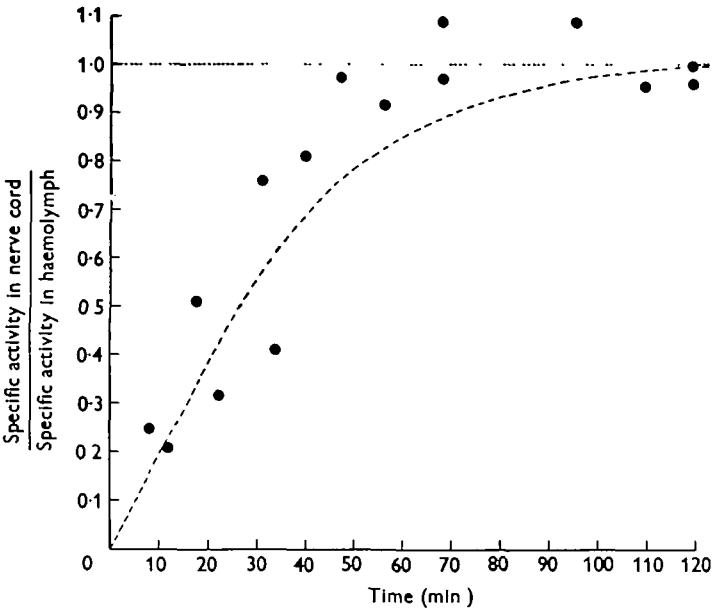

Fig. 2. Changes in the specific activity of ^{22}Na in the nerve cord, relative to that in the haemolymph, following injection of the radioisotope into the haemolymph. The broken line shows the comparable changes in specific activity measured in the abdominal nerve cord of *Periplaneta americana* (Treherne, 1961).

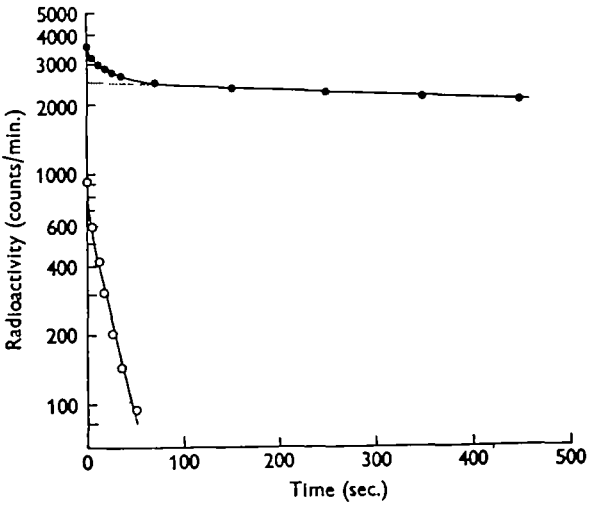

Fig. 3. The escape of ^{22}Na from a nerve cord when washed in non-radioactive physiological solution (closed circles). The nerve cord was made radioactive by the injection of the radioisotope into the haemolymph 4·0 hr. before commencement of the experiment. The fast component of the main curve (open circles) was obtained by subtraction from the straight line extrapolated to zero time.

also show that the rapid fraction in sodium efflux was not affected by the presence of 2,4-dinitrophenol, but that the slow escape of these ions from the nerve cord was significantly reduced in the presence of the poison. These results are similar to those obtained in previous investigations on *Periplaneta* in which the rapid fraction was identified as the extracellular ion fraction (Treherne, 1961*d*, 1962). If the previously obtained figure of 91·8 ml./kg. tissue is used as a measure of the extracellular fluid in the nerve cord then it can be calculated that the sodium concentration of the rapidly exchanging fraction was 212·4 mM./l. This is roughly ten times greater than the measured sodium concentration of the haemolymph. The extracellular concentration also exceeds that of the cellular fraction, which on the basis of a slowly exchanging fraction of 69·5 % can be calculated to be in the region of 86·3 mM./l.

Table 3. *Data from experiments on efflux of ^{22}Na from nerve cords washed in non-radioactive physiological solution*

(These nerve cords were made radioactive by injection of radiosodium into the haemolymph 3 hr. before the experiment.)

Treatment	Serial	Half-time of rapid fraction (mean ± S.E.)	Half-time of slow fraction (mean ± S.E.)	% Na^{22} in rapid fraction	mM./kg. of sodium in rapid fraction
Effluxed in normal solution	1	20·0	868·4	30·4	19·5
	2	21·0	966·5	23·4	
	3	26·0	380·0	35·6	
	4	17·5	480·0	34·9	
	5	14·0	550·0	28·2	
		19·7 ± 2·0 sec.	649·0 ± 113·7 sec.	30·5	
Effluxed in 0·5 mM./l. 2,4-dinitrophenol	6	19·0	1,357·6	22·6	21·9
	7	28·0	1,738·6	47·0	
	8	19·5	1,212·3	29·0	
	9	23·0	1,862·0	33·2	
	10	22·0	1,098·2	39·2	
		22·3 ± 1·6 sec.	1,453·7 ± 148·6 sec.	34·3	

The above calculation on the concentrations of the rapidly exchanging sodium in the nerve cord is based on the assumption that the surface contamination does not contribute appreciably to this fraction. This assumption seems to be justified for the abdominal nerve cord of the cockroach (Treherne, 1962), but there is no evidence that this is the case for *Carausius*. An attempt was made to estimate the extent of the surface contamination by ^{22}Na in the nerve cord of this insect by comparing the amount of the isotope picked up during an approximately 1·0 sec. exposure with that contained in the rapid fraction after a prolonged exposure to the isotope. The results shown in Table 4 give a mean value of 1·04 mM./kg. nerve cord tissue as the amount of radioisotope associated with the cord following a brief exposure to the radioactive solution. This represents only about 5 % of the rapidly exchanging radiosodium so that it is reasonable to assume that the greater part of this fraction is contained within the nerve cord.

Using the calculated half-time of 19·7 sec. for the rapidly exchanging sodium fraction (Table 3) it is possible to arrive at an estimate of the transfer constant (in the direction extracellular fluid → external medium) from the following relation:

$$k_{out} = 0{\cdot}693/t_{0{\cdot}5} = 0{\cdot}0351 \text{ sec.}^{-1}.$$

Table 4. *The sodium content of isolated nerve cords after being dipped for* 1·0 *sec. in the radioactive physiological solution*

Serial	Sodium content (mM./kg. tissue)	Mean
1	1·32	1·04 mM./kg.
2	0·49	
3	0·75	
4	1·11	
5	1·51	

The calculation of the true transfer constant, K_{out}, requires a knowledge of the surface/volume ratio of the system studied:

$$K_{out} = \frac{k_{out}}{A/V},$$

where A and V are the area and volume of the system. An attempt was made to estimate the ratio A/V from measurements made on isolated nerve cords. Considering the ganglia as oblate spheroids and the connectives as cylinders it was possible to estimate the total area of the nerve cord as 56·47 mm.^{2} and the average water content as 0·993μl. Taking the extracellular water as 15·16% of the whole (§2) it is thus possible to roughly estimate the ratio surface/extracellular volume as:

$$A/V = \frac{0{\cdot}5647}{0{\cdot}1516 \times 9{\cdot}93 \times 10^{-4}} = 3752{\cdot}1 \text{ cm.}^{-1}.$$

The true transfer constant then becomes:

$$K_{out} = \frac{0{\cdot}0351}{3752{\cdot}1} = 0{\cdot}35 \times 10^{-6} \text{ cm. sec.}^{-1}.$$

The transfer constant K_{in} (in the direction external medium→extracellular space) can be calculated from the relation:

$$\frac{K_{in}}{K_{out}} = \frac{Na_{extracellular}}{Na_{out}}$$

where $Na_{extracellular}$ and Na_{out} are the sodium concentrations in the extracellular fluid and external medium respectively. Using the calculated value for the extracellular sodium concentration (212·4 mM./l.) and the measured haemolymph values (20·1 mM./l.) the approximate value for true transfer constant becomes:

$$K_{in} = \frac{212{\cdot}4}{20{\cdot}1} . 9{\cdot}35 \times 10^{-6} = 9{\cdot}86 \times 10^{-5} \text{ cm. sec.}^{-1}.$$

Table 5 shows the results of experiments on the efflux of labelled sodium from isolated nerve cords which were loaded *in vitro* by soaking in the solution containing ^{22}Na ions for 1 hr. In one batch of experiments 0·5 mM./l. of 2,4-dinitrophenol was added to the solution before the soaking period. As with the experiments in which the nerve cords were loaded with ^{22}Na *in vivo* the half-time for the rapid fraction remained the same, the slow escape being reduced by the presence of the poison molecules. These results also show quite clearly that the presence of the 2,4-dinitrophenol during the soaking period greatly reduced the amount of sodium in the rapidly exchanging ion fraction.

Table 5. *Data from experiments on the efflux of ^{22}Na from nerve cords which were made radioactive by soaking for 1·0 hr. in normal solution or in one containing 0·5 mM./l. 2,4-dinitrophenol*

Treatment	Serial	Half-time of rapid fraction (sec.)		Half-time of slow fraction (sec.)		Sodium content of rapid fraction (mM./kg. tissue)	
Soaked in normal ^{22}Na solution	1	26·0	22·4 ± 1·4	720·0	618·0 ± 34·9	10 74	11·50 ± 1·19
	2	25·0		500·0		14·93	
	3	19 0		670·0		9·04	
	4	20·0		620·0		13·64	
	5	22·0		570·0		9·15	
Soaked in ^{22}Na solution containing 0·5 mM./l. 2,4-DNP	6	19·6	20·0 ± 0·3	1100·0	1244·0 ± 67·3	2·92	2·67 ± 0·21
	7	19·7		1360·0		2·29	
	8	20·0		1320·0		2·07	
	9	21·0		1060·0		2·97	
	10	19·5		1380·0		3·13	

(5) *The effect of a metabolic inhibitor on sodium uptake*

In these experiments the uptake of ^{22}Na by isolated nerve cords was compared as between those soaked in normal physiological solution and those exposed in addition to 0·5 mM./l. 2:4- dinitrophenol. The excised nerve cords were pre-treated for 5 min. in normal or poisoned non-radioactive solution. Figure 4 illustrates the specific activity changes of the sodium within the nerve cord relative to that in the external solution. Despite the considerable individual variation these results indicate that the rate of mixing of labelled sodium ions in the nerve cord tissues took place more slowly in the presence of the poison molecules.

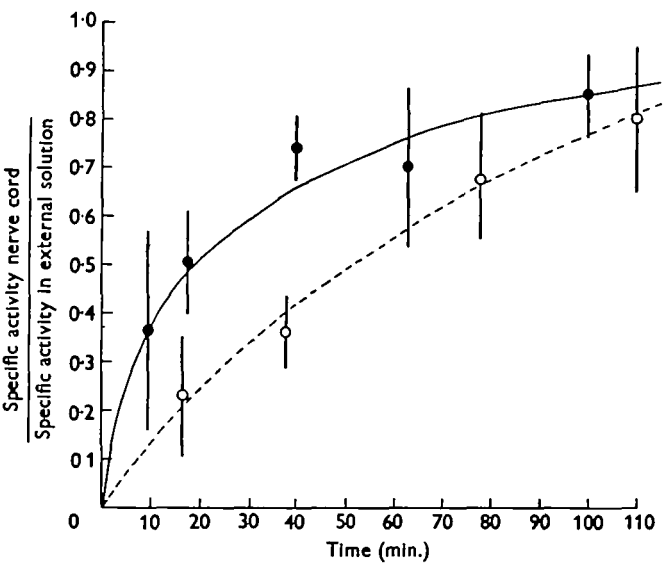

Fig. 4. Changes in the specific activity of ^{22}Na, relative to the external medium, in isolated nerve cords soaked in radioactive physiological solution. The closed circles represent the uptake of ^{22}Na with normal solution; the open circles that measured with a solution containing 0·5 mM./l. 2,4-dinitrophenol. The vertical lines represent the extent of twice the standard error of the means.

In view of the variation experienced in the above experiments some direct measurements were made on the uptake of radiosodium by isolated nerve cords. For this purpose the excised nerve cords were pre-treated for 5 min. by soaking in non-radioactive solutions. In half these experiments this solution also contained 0·5 mM./l. 2,4-dinitrophenol. The nerve cords were then transferred to solutions in which the sodium was labelled with ^{24}Na. At varying intervals after this the cords were removed from the radioactive solution, quickly washed in non-radioactive solution, blotted and then their radioactivity was measured with a GM tube. The isolated cords were then returned to the radioactive solution before being assayed again. The effect of the metabolic inhibitor on this process is illustrated in Fig. 5. These results show that the effect of the poison is on the initial rapid uptake of the isotope, although the slope of the line for the subsequent uptake appears to be steeper in the normal as compared with the poisoned preparations.

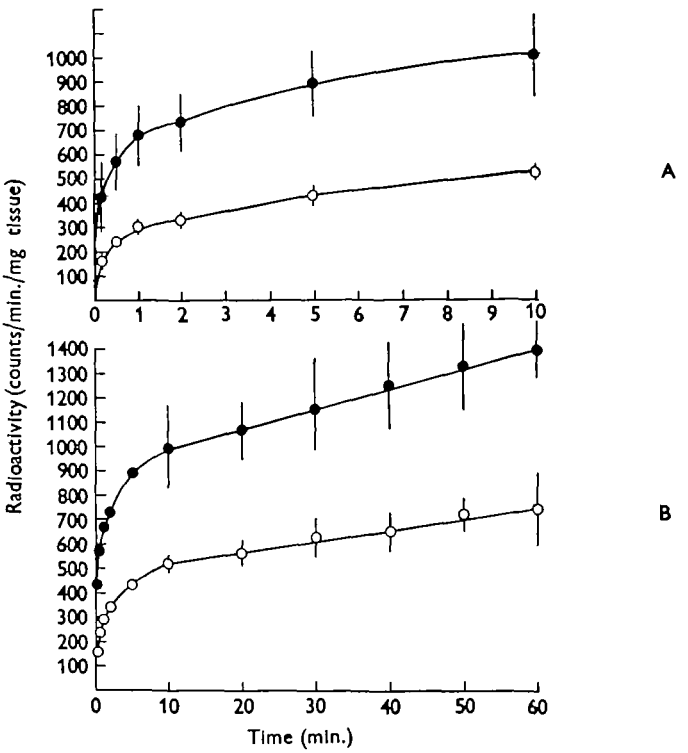

Fig. 5. The uptake of ^{24}Na by isolated nerve cords soaked in radioactive physiological solution. In these experiments nerve cords were returned to the radioactive solution between successive measurements. The closed circles represent the uptake in normal solution; the open circles that measured in solution containing 0·5 mM./l. 2,4-dinitrophenol. Each point represents the mean of six determinations, the vertical lines being the extent of twice the standard error. The upper figure (A) illustrates the initial uptake of ^{24}Na; while the lower one (B) shows the uptake obtained in the complete experiment.

(6) *Potassium efflux experiments*

The efflux of ^{42}K was studied in experiments in which the nerve cord was made radioactive by the injection into the haemolymph or by soaking isolated cords in a

solution containing labelled potassium ions. Figure 6 shows that, as for sodium, the efflux of ^{42}K can be represented as a two-stage process. The results of the *in vivo* experiments showed some variation in the percentage of the rapidly exchanging potassium fraction (Table 6).

Table 6. *Data from experiments on the efflux of ^{42}K from nerve cords washed in non-radioactive solution*

(The nerve cords were made radioactive by injection of ^{42}K into the haemolymph 4 hr. before the commencement of the experiment.)

Serial	Half-time of rapid fraction (sec.)		Half-time of slow fraction (sec.)		% of ^{42}K in rapid fraction
1	22·0		1000·0		41·0
2	14·0		440·0		21·9
3	17·0	16·4 ± 1·50	660·0	577·0 ± 145·4	15·1
4	15·0		1011·0		32·4
5	14·9		674·0		28·3

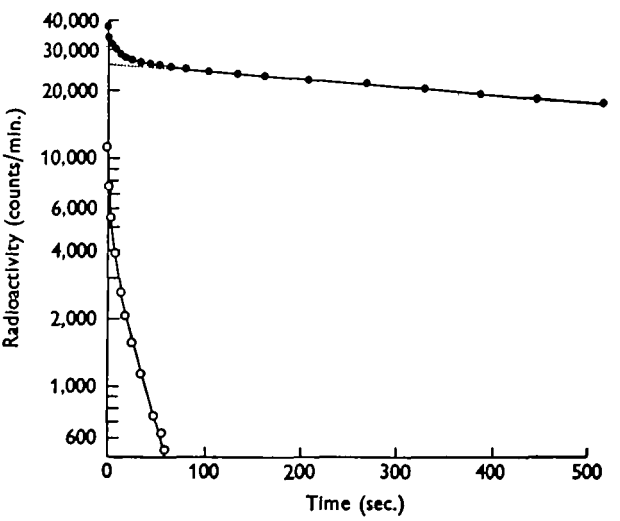

Fig. 6. The escape of ^{42}K from a nerve cord which had been made radioactive by soaking in a solution containing the radioisotope for 1·0 hr. before the commencement of the experiment (closed circles). The fast component (open circles) was obtained by extrapolation of the straight line to zero time.

The above variation resulted partly from the low specific activity of the ^{42}K used in these experiments. The further experiments, summarized in Table 7, were, therefore, carried out by soaking the isolated nerve cords in solutions containing radioactive potassium, thus avoiding the dilution of the isotope obtained on injection into the haemolymph. In some experiments isolated nerve cords were soaked for 1·0 hr. and then effluxed in normal solution; in the second batch the nerve cords were soaked for 1·0 hr. and then effluxed in a solution containing 0·5 mM./l. 2,4-dinitrophenol and 2·0 mM./l. iodoacetic acid. It will be seen from Table 7 that the rate of efflux of both the rapid and the slow ion fractions was unaffected by the presence of the poisons. The

concentration of the ^{42}K in the rapid fraction was also not affected by the presence of the poison molecules. The concentration of the ^{42}K which had been accumulated in the slow fraction during the experimental period was, however, reduced by the presence of the 2,4-dinitrophenol and the iodoacetic acid.

Table 7. *Data on escape of ^{42}K from nerve cords loaded* in vitro *for 1 hr. before commencement of the experiment*

Treatment	Serial	Half-time of rapid fraction (sec.)		Half-time of slow fraction (sec.)		Potassium content of rapid fraction (mM./kg. tissue)		Potassium content of slow fraction (mM./kg. tissue)	
Loaded and effluxed in normal solution	1	15·0	18·8 ± 1·2	1320·0	943·0 ± 110 8	8·55	9·81 ± 0·62	24·08	17·56 ± 1·83
	2	17 5		812 0		8·65		18·99	
	3	22 0		660 0		11·60		15·81	
	4	19·5		1003·0		9·46		14·96	
	5	20 0		920 0		10·80		13·98	
Loaded and effluxed in solution containing 0·5 mM./l. 2,4-DNP and 2·0 mM./l. iodoacetic acid	6	20·0	20·4 ± 1·1	580·0	1008·0 ± 128·1	11 1)	10·62 ± 0 38	6 55	9·57 ± 1·75
	7	23·0		1240·0		11 19		11·36	
	8	21·0		850·0		9·19		9 19	
	9	21·5		1140·0		10·52		10·15	
	10	16·5		1230·0		11·02		10·58	

(7) *The effect of metabolic inhibitors on potassium uptake*

The results of the experiments illustrated in Fig. 7 show that the presence of 0·5 mM./l. 2,4-dinitrophenol and 2·0 mM./l. iodoacetic acid did not significantly affect the initial stages of the uptake of ^{42}K in these isolated preparations.

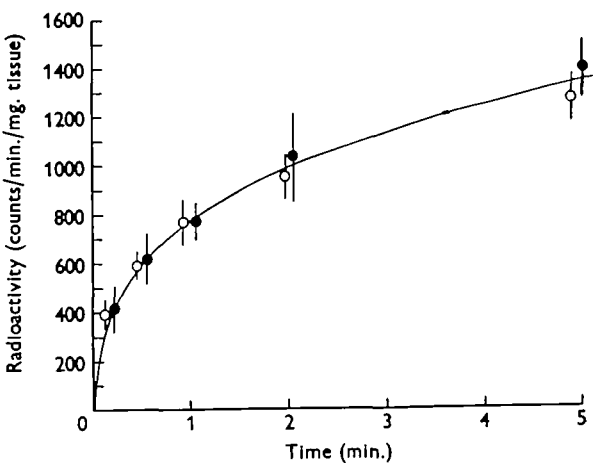

Fig. 7. The uptake of ^{42}K by isolated nerve cords soaked in radioactive physiological solution. The radioactivity of the nerve cords was measured by removing them from the medium between successive periods of exposure to the experimental solution. The closed circles represent the uptake of ^{42}K in normal solution; the open circles that measured in solution containing 0·5 mM./l. 2,4-dinitrophenol and 2 0 mM./l. iodoacetic acid. Each point represents the mean of six determinations, the vertical lines being the extent of twice the standard error.

(8) *The efflux of calcium ions*

Isolated nerve cords were loaded with ^{45}Ca by soaking them for 1 hr. in a solution containing this radioisotope. The subsequent efflux of the labelled ions was studied by washing them in inactive solution (Fig. 8). In one batch of radioactive nerve cords the efflux was carried out in the presence of 0·5 mM./l. 2,4-dinitrophenol, whilst in another batch both the loading and the efflux were carried out in the presence of this poison. These results are summarized in Table 8. The presence of the metabolic inhibitor did not produce any dramatic effects either on the rate of efflux or on the concentrations of the rapid or slowly exchanging calcium fractions in the nerve cord.

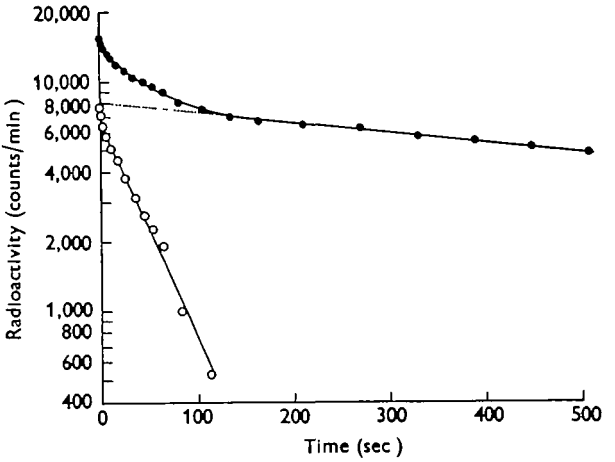

Fig. 8. The escape of ^{45}Ca from a nerve cord when washed in non-radioactive physiological solution (closed circles). The nerve cord was made radioactive by soaking it for a period of 1·0 hr., before the commencement of the experiment, in a solution containing the radioisotope. The fast component was obtained by extrapolation of the straight line to zero time (open circles).

Table 8. *Efflux of ^{45}Ca from isolated nerve cords previously loaded in vitro for 1 hr. when washed in non-radioactive solution*

Treatment	Serial	Half-time of rapid fraction (sec.)		Half-time of slow fraction (sec.)		Calcium content of rapid fraction (mM./kg. tissue)	
Loaded and effluxed in normal solution	1	31·0	25·4 ± 2·0	940 0	890·0 ± 28·5	1·60	2·06 ± 0·18
	2	26 0		770·0		1·60	
	3	21·0		850·0		2·25	
	4	28 0		970·0		2 66	
	5	21·0		920·0		2·17	
Loaded in normal solution and effluxed in one containing 0 5 mM./l. 2,4-DNP	6	23·0	19 2 ± 1·0	640·0	604·0 ± 60·5	2 52	2·56 ± 0·13
	7	19·0		820·0		2·37	
	8	19·0		440·0		2·22	
	9	18 0		500 0		2·96	
	10	17·0		620·0		2·75	
Loaded and effluxed in solutions containing 0 5 mM./l. 2,4-DNP	11	28 5	23·9 ± 1·2	1080·0	808·8 ± 78·1	1·70	2·23 ± 0 35
	12	23·0		650·0		1 62	
	13	23·0		770·0		3 47	
	14	22·0		664·0		2·51	
	15	23·0		880 0		1 87	

The efflux of magnesium ions

The escape of magnesium ions from the nerve cord was studied by loading the isolated preparations for 1 hr. in physiological solution containing ^{28}Mg and studying the efflux in non-radioactive solution. The data from these experiments are summarized in Table 9. The mean concentration of the magnesium ions in the fast fraction was 13·8 mM./kg. tissue.

Table 9. *Efflux of ^{28}Mg from nerve cords which were soaked in radioactive solution for 1 hr. before commencement of the experiment*

Serial	Half-time of rapid fraction (sec.)		Half-time of slow fraction (sec.)		Magnesium content of rapid fraction (mM./kg. tissue)	
1	24·0		930·0		14·2	
2	23·0		560 0		10·7	
3	22·0	22·4 ± 0·8	540·0	721·0 ± 74·9	13 9	13·8 ± 0·8
4	23·5		815·0		14·5	
5	19·5		760 0		15·5	

(9) *Experiments on the uptake and efflux of chloride ions*

Figure 9 illustrates the efflux of ^{36}Cl from a nerve cord which had been soaked in a solution containing the radioisotope for 1 hr. The mean concentration of chloride in the rapidly exchanging fraction was found to be 31·4 mM./kg. nerve cord tissue (Table 10). These results should be considered in relation to those carried out on the uptake of ^{36}Cl obtained in approximately 1·0 sec. exposures to the physiological solution (Table 11). Unlike those carried out with ^{22}Na these results with radiochloride showed that an appreciable proportion, 19·6 mM./kg., was associated with the nerve cord following a brief exposure to the experimental solution. If this effect resulted entirely from surface contamination then the true rapidly exchanging fraction within the cord would only be about 11·8 mM./kg. tissue. It is thus only possible to make the rather unsatisfactory statement that the true value for the concentration of the rapidly exchanging chloride fraction lies between 11·8 and 31·4 mM./kg. nerve cord tissue.

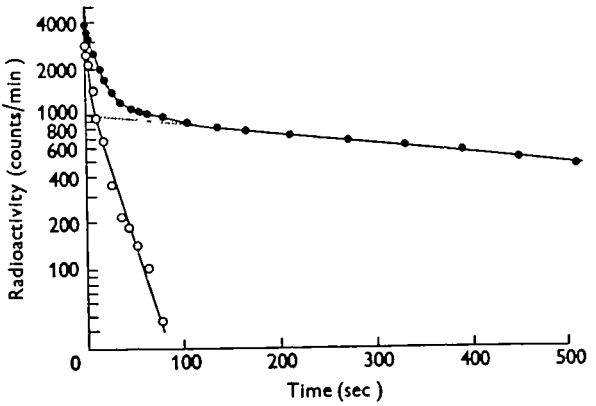

Fig. 9. The escape of ^{36}Cl from a nerve cord which was loaded *in vitro*, by soaking it in radioactive solution for 1·0 hr. before the commencement of the experiment (closed circles). The fast component (open circles) was obtained by extrapolation of the straight line to zero time.

Table 10. *Efflux of ^{36}Cl from nerve cords loaded by soaking in radioactive solution for 1·0 hr.*

Serial	Half-time of rapid fraction (sec.)		Half-time of slow fraction (sec.)		Chloride content of rapid fraction (mM./kg. tissue)	
1	19·0		460·0		25·1	
2	17·5		339 0		35·1	
3	18·0	17·4 ± 0·7	420·0	360·6 ± 38·8	38·0	31·4 ± 2·7
4	17·7		234·0		25 1	
5	15·0		350·0		33 8	

Table 11. *Chloride content of nerve cord tissues following a 1·0 sec. exposure to the radioactive solution*

Serial	Chloride content (mM./kg. tissue)	Mean ± S.E.
1	26·4	
2	23 1	
3	19·9	19·6 ± 2·4 mM./kg.
4	15·2	
5	13 2	

Experiments were also carried out to investigate the effects of metabolic inhibitor on the initial uptake of ^{36}Cl into the nerve cord. Isolated nerve cords were soaked for 5 min. in normal physiological solution, or in one containing 0·5 mM./l. 2,4-dinitrophenol, before being transferred to the ^{36}Cl-labelled solution for a period of 2 min. The radioactive nerve cords were quickly washed in normal solution and then blotted before the ^{36}Cl content was determined. The results, which are summarized in Table 12, indicate that the initial uptake of ^{36}Cl was reduced by the presence of the metabolic inhibitor ($P < 0·05$).

Table 12. *Uptake of ^{36}Cl by isolated nerve cords after soaking for 2·0 min. in radioactive physiological solution*

Treatment	Serial	^{36}Cl content (counts/min./mg. tissue)	Mean ± S E.
Normal solution	1	627·0	
	2	502·3	
	3	677·0	613·4 ± 60·1
	4	801·7	
	5	459·2	
Solution containing 0·5 mM./l. 2,4-DNP	6	432·5	
	7	479·7	
	8	328·1	437 6 ± 33·6
	9	520·2	
	10	427·5	

DISCUSSION

The measurements on the ionic content of the central nervous system showed that the concentration of sodium in the nerve cord of *Carausius* (102·4 mM./l.) was very close to that of *Periplaneta americana* (103·2 mM./l.; Treherne, 1961*a*) despite the fact that the concentration of this ion in the haemolymph is almost an order greater

in the latter insect. The potassium content of the nerve cord tissues in *Carausius*, on the other hand, greatly exceeded that of the cockroach (510·3 as against 180·2 mM./l.). This difference can be related to the haemolymph concentration of this ion in the phytophagous insect, for the potassium level in the haemolymph exceeded that of the cockroach by a factor of 2·7 (33·7 as compared with 12·3 mM./l.). With the divalent cations it should be noted that the tissue concentration of magnesium was low whilst that of calcium was high relative to their haemolymph concentrations.

The experiments on the *in vivo* uptake of ^{22}Na by the nerve cord of *Carausius* showed that the changes in the specific activity of the ion within nervous tissues occurred at approximately the same rate as in the cockroach central nervous system, despite the very low level of this ion in the haemolymph of *Carausius*. As the nerve cords of these two insects are of the same order of size these results must indicate a changed permeability to this cation at some stage in its entry into the tissues of the central nervous system of the latter species.

The existence of an appreciable extracellular fraction in the cockroach central nervous system was inferred from the following evidence (Treherne, 1961*d*, 1962):

(1) The efflux of labelled sodium from the isolated nerve cord was found to occur as a two-stage process.

(2) The escape of the rapidly exchanging fraction was unaffected by the presence of metabolic inhibitors, although the efflux of the slowly exchanging sodium (identified as that taking place from the cellular fraction) was reduced under these circumstances.

(3) The rapidly exchanging component was present in desheathed preparations, and was, therefore, not associated with the nerve sheath.

(4) 18·2% of the nerve cord water was found to be accessible to ^{14}C-inulin molecules.

(5) The efflux of triated water occurred as a two-stage process, the rapidly exchanging fraction being of a similar magnitude to the demonstrated inulin space.

(6) Electron micrographs of the nerve cord demonstrated the existence of an appreciable extracellular system, sufficient to accommodate the inulin molecules used in the previous experiments (Smith & Treherne, 1963).

The present experiments on the nerve cord of *Carausius* also demonstrated that the efflux of labelled sodium occurred as a two-stage process: an initial rapid escape, with a half-time of 19·7 sec., giving way to a slower leakage with a half-time of 649·0 sec. As with the nerve cord of *Periplaneta* the slow component of sodium efflux was significantly reduced by the presence of 0·5 mM./l. 2,4-dinitrophenol, the initial rapid escape being unaffected by this metabolic inhibitor. Similarly, only a relatively small proportion of the rapidly exchanging fraction appeared to be associated with the nerve sheath. The measured inulin space of 15·2% of the nerve cord water was lower than for the cockroach central nervous system. These results would, however, seem to indicate an essentially similar state of affairs, as far as the efflux of sodium is concerned, to that prevailing in the cockroach. Thus it seems reasonable to conclude that the slow component in the *Carausius* nerve cord represented the secretion of sodium from the cellular fraction, the initial escape being that of the ions from the extracellular fluid. It has been pointed out that the extrusion of sodium ions from the cellular fraction of the central nervous system of *Periplaneta* appears to be dependent on the presence of external potassium (Treherne, 1961*b*, *c*)

and it was suggested that as in the squid giant axon (cf. Hodgkin, 1958) the efflux is effected by some sort of linked sodium pump. A similar state of affairs may also exist in the nerve cord of *Carausius*, for it has been demonstrated that uptake of ^{42}K into the slowly exchanging fraction is reduced in the presence of metabolic inhibitors (Table 7).

Table 13. *The calculated concentrations of the rapidly exchanging ion fractions in the nerve cord of* Carausius

(The estimates of the extracellular concentration are based on the measured inulin space of the nerve cord.)

Ion	Type of experiment	Concentration in haemolymph or external solution (mM./l.)	Tissue content of rapidly exchanging ion fraction (mM./kg. tissue)	Calculated extracellular concentration (mM./l.)	Ratio: (extracellular concentration)/(external concentration)
Na	*in vivo*	20·1	19·5	212·4*	10·6
	in vitro	15·0	11·5	78·7†	5·2
	in vitro (poisoned)	15·0	2·7	18·5†	1·2
K	*in vitro*	18·0	9·8	67·1†	3·7
	in vitro (poisoned)	18·0	10·6	72·5†	4·0
Ca	*in vitro*	7·5	2·1	14·4†	1·9
	in vitro (poisoned)	7·5	2 2	15·0†	2·0
Mg	*in vitro*	50·0	13·8	94·4†	1·9
Cl	*in vitro*	133·0	11·8–31·4	80·7–214·9†	0·61–1 6

* Based on *in vivo* inulin space of 91·8 ml./kg. tissue.
† Based on *in vitro* inulin space of 146·1 ml./kg. tissue (cf. Table 2).

If the concentrations of the rapidly exchanging ion fractions are related to the measured inulin space in the ventral nerve cord of *Carausius* (Table 2) then it is possible to arrive at an approximate estimate of the extracellular concentrations of these ions (Table 13). In the cockroach it was suggested that the relatively high concentrations of cations in the extracellular fluid resulted from a Donnan equilibrium with the haemolymph (Treherne, 1962). It is evident that no such simple relation appears to exist for the distribution of the ions between the extracellular fluid and the haemolymph in *Carausius morosus*.

The most obvious departure from a simple Donnan equilibrium is the high concentration of sodium ions in the extracellular fraction relative to the haemolymph (the ratio being 10·6) as compared with the other cations. In the *in vitro* preparation the ratio of the extracellular to the external sodium concentrations fell to 5·2, due partly to enlargement of the inulin space in the isolated preparation. In the presence of 2,4-dinitrophenol the ratio fell still further to 1·2. The obvious dependence of the high extracellular sodium concentration on aerobic metabolism clearly suggests some sort of secretion of sodium ions into the extracellular fluid. This supposition was confirmed by the experiments demonstrating the effects of the metabolic inhibitor on the initial rapid uptake of ^{22}Na and ^{24}Na by isolated nerve cords (Figs. 4 and 5). These experiments also help to explain the observation, which was discussed earlier, that

changes in the specific activity of ^{22}Na within the nerve cord of *Carausius* took place as rapidly as in *Periplaneta* although the concentration gradient between the haemolymph and the nerve cord tissues was much steeper in the former species.

Unlike the experiments on the effects of metabolic inhibitors on the rapidly exchanging sodium fraction the equivalent experiments with ^{42}K and ^{45}Ca did not produce evidence for any secretion of these cations into the extracellular fluid. It therefore seems reasonable to assume that the concentrations of these cations and of sodium in the poisoned preparations result from a passive distribution between the external medium and the extracellular fluid. It is now relevant to inquire whether under these conditions the ions can be considered to be distributed according to a Donnan equilibrium. The presence of such a distribution is usually postulated in cells and tissues from a consideration of the ratios of the chloride and the cation concentrations between the outside medium and the compartment containing the indiffusible anion groups. In the present experiments this is impossible to establish because of the uncertainties involved in defining the rapidly exchanging chloride fraction. However, the following distribution of cations would be expected from the above considerations in the case of a Donnan equilibrium between the external medium and the extracellular fluid:

$$\frac{Na_{extracellular}}{N_{out}}\ (poisoned) = \frac{K_{extracellular}}{K_{out}} = \left(\frac{Ca_{extracellular}}{Ca_{out}}\right)^{\frac{1}{2}} = \left(\frac{Mg_{extracellular}}{Mg_{out}}\right)^{\frac{1}{2}}$$

The observed ratios were as follows:

$$\frac{Na_{extracellular}}{Na_{out}}\ (poisoned) = 1{\cdot}23,$$

$$\frac{K_{extracellular}}{K_{out}} = 3{\cdot}73,$$

$$\left(\frac{Ca_{extracellular}}{Ca_{out}}\right)^{\frac{1}{2}} = 1{\cdot}37,$$

$$\left(\frac{Mg_{extracellular}}{Mg_{out}}\right)^{\frac{1}{2}} = 1{\cdot}37.$$

It seems clear from these ratios that the extracellular concentrations of sodium ions in poisoned preparations and of calcium and magnesium ions could be the results of a Donnan equilibrium with the haemolymph. The potassium ions, however, depart markedly from the concentration which would be expected in the case of a Donnan equilibrium. The concentration of potassium ions in the extracellular fluid is independent of the concentration in the slowly exchanging fraction (Table 7) and no metabolic processes seem to be involved in the uptake of these ions into the extracellular compartment (Fig. 7). One possibility which remains is that some potassium in the extracellular fluid associates with specific anion groups. Such an association would presumably be analogous to ion 'binding', so that it might be expected that the mean activity coefficient of the potassium ions would be reduced in the extracellular fluid.

The rapid initial uptake of chloride ions by the isolated nerve cord was shown to be reduced in the presence of 0·5 mM./l. 2,4-dinitrophenol (Table 12). This effect could have resulted from the poisoning of the process involved in the uptake of sodium ions,

for it might be expected that chloride ions would passively follow the active secretion of the sodium into the extracellular fluid. Such an uptake of chloride ions into an extracellular system containing fixed or indiffusible anion groups would again tend to obscure the Donnan effect with the haemolymph.

The extracellular compartment of the nerve cord of *Carausius* appears, from the relatively rapid exchanges taking place with the haemolymph, to be a rather 'leaky' system. The pumping of sodium ions into the extracellular fluid must, therefore, be a very rapid process. This process is, for example, of a different order of magnitude from the outward secretion of sodium ions from the cells into the extracellular fluid of the central nervous system. It is relevant to compare this process with other sodium-transporting systems in insects. The most detailed investigations of sodium transport in insects appear to be those carried out on the anal papillae of the larvae of *Aedes aegypti* (Treherne, 1954; Stobbart, 1959, 1960). The calculated permeability constant, k_{out} (in the direction haemolymph → external medium), for fed larvae maintained in 2·0 mM./l. Na was 0·0761 hr.$^{-1}$ or $2{\cdot}11 \times 10^{-5}$ sec.$^{-1}$ (Stobbart, 1959). To estimate K_{out}, the true permeability constant of the anal papillae, it is necessary to know the effective surface/volume ratio of the system. A rough estimate of this ratio can be made by comparing the haemolymph volume of 4th-stage larva (measured by weighing that squeezed from individual insects) with the surface area of the papillae (in which these organs were considered as cylindrical structures). The mean result of 2·053 mm.^{2} for 128 μl. of haemolymph gave an approximate surface volume/ratio of 1·604 cm.$^{-1}$. It seems legitimate to use the haemolymph for the volume in this ratio, for it has been shown that the exchanges between it and the external medium approximate to those taking place in a two-compartment system (Treherne, 1954). The constant K_{out} then becomes $1{\cdot}31 \times 10^{-5}$ cm. sec.$^{-1}$ and, with a haemolymph concentration of 116·0 mM./l. Na (Stobbart, 1959), K_{in} is approximately $7{\cdot}60 \times 10^{-4}$ cm. sec.$^{-1}$. K_{in} for the exchanges taking place in the nerve cord of *Carausius* has already been estimated to be about $9{\cdot}86 \times 10^{-5}$ cm. sec.$^{-1}$. It is apparent, therefore, that the transport of sodium ions into the extracellular fluid of the central nervous system of *Carausius* is taking place at less than the rate demonstrated for the uptake of these ions by the anal papillae of *Aedes* larvae.

The demonstrated transport of sodium ions into the extracellular fluid presumably results from the activities of the cellular layer, or perineurium, which is associated with the connective tissue sheath of the insect central nervous system (cf. Smith & Treherne, 1963). The ability of the nerve of *Carausius* to continue functioning when portions of the sheath were removed (Treherne, 1965) suggests that other glial cells may also be involved in sodium movements into the extracellular system, for in the cockroach desheathing has been shown to cause removal or damage to the perineurium (Twarog & Roeder, 1956).

There has been in the past some speculation as to the means by which the axons of phytophagous insects are able to function in haemolymph containing very low concentrations of sodium ions and relatively high levels of other cations. The present investigation has shown that the cells of the central nervous system of *Carausius* function in a medium with a relatively high sodium content. Table 14 contains the estimated extracellular and cellular concentrations of the various cations used in this investigation. It should be emphasized that the figures for potassium, calcium and

Table 14. *The estimated cellular and extracellular ion concentrations in the nerve cord*

(The values for sodium are based on *in vivo* studies, those for the remaining cations involve the extrapolation of *in vitro* results to the tissue concentrations shown in Table 1.)

Cation	Concentration in haemolymph (mM./l.)	Estimated concentration in extracellular fluid (mM./l.)	Estimated concentration in cellular water (mM./l.)
Na	20·1	212·4	86·3
K	33·7	124·5	555·8
Ca	6·4	12·2	61·8
Mg	61·8	117·4	10 7

magnesium are only approximate for they involve the extrapolation of data from *in vitro* studies to those made on the intact nerve cord. The resting potential of many excitable cells has been shown to be related to the external and internal potassium concentrations by the Nernst equation:

$$E_K = \frac{RT}{zF} \log_e \frac{K_i}{K_0},$$

where E_K is the potassium equilibrium potential, z the valency, F the Faraday, R the gas constant, T the absolute temperature and K_i and K_0 the internal and external potassium concentrations. Applying this equation to the estimated extracellular and cellular potassium concentrations in Table 14 gives a value of $-37{\cdot}1$ mV for the resting potential. The potassium equilibrium potential should strictly be related to the activities of the ions within and outside the cells rather than to the ratio of their chemical concentrations. The activity of potassium in the axoplasm of squid is known to be equivalent to that in free solution (Hinke, 1961). If this is also the case for the axons of *Carausius* then it is possible that the resting potential would be greater than that estimated on the basis of the concentration difference for, as has already been discussed, the possibility cannot be eliminated that the activity coefficient of the potassium in the extracellular phase may be reduced. The calculated potassium equilibrium potential is low compared with the measured resting potentials in excitable cells of some other insects which have been investigated. The resting potential for locust muscle, for example, was about 63·2 mV (Hoyle, 1955) and for cockroach axons 77·0 mV (Yamasaki & Narahashi, 1959). The calculated value of 37·1 mV for *Carausius* axons is, however, close to the observed resting potential of 41·0 mV for the muscle fibres of this insect (Wood, 1957).

Using the value contained in Table 14 the predicted sodium equilibrium potential resulting from the entry of these ions along the electrochemical gradient during the active phase will be given by the relation:

$$E_{Na} = \frac{RT}{zF} \log_e \frac{Na_0}{Na_i},$$

where Na_0 and Na_i are the concentrations inside and outside the cell. This would be equivalent to an equilibrium potential of 22·3 mV, which is of the same magnitude as the 22·0 mV reversed potential recorded during the action potential of cockroach axons (Yamasaki & Narahashi, 1959). These considerations will serve to explain the

observation that removal of the very low concentration of sodium ions in the external medium resulted in a rapid conduction block in the intact nerve cord of *Carausius* (Treherne, 1965). In the complete absence of external sodium ions the secretory pump would not be able to maintain the high extracellular level of the ion, so that the concentration gradient between the extracellular fluid and the axoplasm would be abolished.

The conduction processes in the nerve cord of *Carausius* have been shown to be dependent upon the presence of external magnesium ions as well as sodium ions Treherne, 1965). Such an effect could result from some secondary effects of the divalent ions on the axon membranes within the nerve cord. It is also possible, however, as with the muscle fibres of this insect (Wood, 1957) and of the crab (Fatt & Katz, 1953; Fatt & Ginsborg, 1958), that the action potential could result from an inward movement of the divalent ion. In the event of such an influx of both sodium and magnesium ions it can be calculated (using the Nernst equation, and from the ratio of 11·0 shown in Table 14) that the magnesium ions alone would only contribute an equilibrium potential of about 29·6 mV. The involvement of the divalent ions would thus only produce a slight increase in the mean potential as compared with that resulting from the movement of sodium ions alone. The increased magnesium conductance would, however, contribute appreciably to the total ionic current carried during the action potential. These estimates of the possible effects of the high concentration of magnesium ions in the extracellular fluid are made on the assumption that the activity coefficients are the same in this phase and within the axons. Any reduction in the activity of the cation in either phase would result in a departure from the estimated magnesium equilibrium potential. If, as is the case for calcium ions which move in the axoplasm at less than 1/30 the rate in free solution (Hodgkin & Keynes, 1957), there is a reduced activity within the axons in this insect then it is possible that the potential resulting from any inward movement of magnesium ions could be greater than that indicated above.

SUMMARY

1. The distribution and exchange of inorganic ions between the central and the haemolymph has been studied in the stick insect, *Carausius morosus*, by flame photometry and radioactive tracers.

2. The exchanges of labelled ions show rapid and slow components which correspond to extracellular and intracellular compartments within the central nervous system.

3. The uptake of sodium from the haemolymph and its concentration in the extracellular fluid is reduced in the presence of metabolic inhibitors.

4. The distribution between haemolymph and extracellular fluid of calcium and magnesium, and also of sodium in poisoned preparations, conforms to a Donnan equilibrium. The distribution of potassium, even in poisoned preparations, does not conform and it is suggested that the activity of this ion may be lower than in free solution.

5. The concentration of magnesium is appreciably greater in the extracellular than in the intracellular compartment. The possible role of magnesium in nervous transmission in this insect is discussed.

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