SODIUM EFFLUX FROM THE CENTRAL NERVOUS CONNECTIVES OF THE COCKROACH

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INTRODUCTION

In his experiments on locust peripheral nerve Hoyle (1953) showed that whereas conduction remained unimpaired in high-potassium solutions in intact nerves, the injection of the same solution beneath the sheath resulted in a rapid conduction block. Some years later Twarog & Roeder (1956) found that the time to block the conduction at the level of the 4th abdominal ganglion of the cockroach by a solution containing 140 mM/l of potassium was reduced from 25 min to 1 or 2 min by de-sheathing. Similarly, perfusion of the intact ganglion by a sodium-free solution was ineffective if the sheath was intact, whereas the conduction in the large fibres was blocked within 30 sec if this sheath was removed. These facts were interpreted as a demonstration that the sheath acted as a diffusion barrier to ions. Yamasaki & Narahashi (1960) also interpreted the ineffectiveness of acetylcholine on sheathed ganglia of the cockroach in terms of an extremely impermeable diffusion barrier.

Unexpectedly, radioisotope experiments have shown that the exchange of ^{14}C-labelled sugars (Treherne, 1960), of sodium and potassium ions (Treherne, 1961 *a*) and of quaternary ammonium salts (Eldefrawi & O'Brien, 1967) takes place relatively rapidly between the haemolymph and the abdominal nerve cord of *Periplaneta*. Partial de-sheathing of isolated ganglia was also shown to have no significant effect on the rate of efflux of ^{24}Na from the system (Treherne, 1961 *c*, *d*). It was later suggested that depolarization observed in de-sheathed preparations (as compared with intact nerves) in high external potassium concentration might result from the disruption by de-sheathing of a Donnan equilibrium which normally exists between the extracellular space and the external solution (Treherne, 1962 *a*, *b*). A similar interpretation was also given by Pichon & Boistel (1967) in their microelectrode analysis of the resting and action potentials of the cockroach giant axons.

More recently, a microelectrode analysis of the effect of high-potassium solutions on intact, stretched and de-sheathed connectives of *Periplaneta* showed that in stretched and de-sheathed connectives there is a very good agreement between theoretical predictions (free diffusion along a complex pathway) and experimental results (Treherne *et al.* 1970). In 'intact' preparations (i.e. sheathed and unstretched), however, the half time for the depolarization of the giant axons in high potassium was found to be considerably longer than theoretically expected. This lengthening was

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accompanied by the building up of an inwardly positive diffusion potential which was called an 'extraneuronal' potential, because of its extra-axonal origin. The theory had thus to be modified by the addition, in series with the previous extracellular free diffusion pathway, of a peripheral barrier, probably located at the perineurial level (Treherne et al. 1970; Pichon & Treherne, 1971). This barrier appeared to be very sensitive to stretching or drying (Pichon & Treherne, 1970). The extraneuronal potentials have been explained in terms of diffusion across this barrier (Pichon, Moreton & Treherne, 1971).

The present experiments have been carried out as an attempt to establish a correlation between the state of the preparation, as indirectly estimated from the size of the extraneuronal potentials in high-potassium solutions, and the efflux of ^{22}Na ions from intact or de-sheathed connectives of Periplaneta americana. They have been performed on short lengths (2 mm) of the penultimate connectives, as were the preceding electrophysiological experiments (Treherne, et al. 1970; Pichon & Treherne, 1970; Pichon et. al. 1971; Pichon & Treherne, 1971). In the discussion an attempt is made to analyse the functional properties of the system in terms of different interconnected compartments and to compare the behaviour of this system in steady-state and non-steady-state conditions.

MATERIALS AND METHODS

The adult male Periplaneta americana was used exclusively in these experiments as it possesses a nerve cord which is relatively free from the associated fat body. Measurement of sodium effluxes and electrical recordings were made only from the connectives between the 4th and 5th abdominal ganglia, that, is the penultimate connectives. All experiments were performed at room temperature ranging from 18 to 24 °C.

Saline. The physiological solution was that devised by Yamasaki & Narahashi (1959) and it had the following composition: 214 mM/l Na$^+$, 3·1 mM/l K$^+$, 1·8 mM/l Ca^{2+}, 216·9 mM/l Cl$^-$, 0·2 mM/l H$_2$PO$_4^-$ and 1·8 mM/l HPO$_4^{2-}$.

Loading with ^{22}Na. To load the connectives radioactively isolated ligatured nerve cords were soaked in physiological solution in which ^{22}Na had been incorporated as ^{22}NaCl. (In some experiments the penultimate connectives were de-sheathed before loading with ^{22}Na.) Nerve cords were loaded in vitro rather than by injection of ^{22}Na into the haemolymph in order to get sufficiently high specific activity of ^{22}Na in the nerve to enable measurable counts to be obtained in the 'washing' solution over a period of 35 min. Since Treherne (1961a) had found that it required about 1·5 h for nerve cords to come to equilibrium with the bathing solution when they were loaded with ^{24}Na, in this series of experiments nerve cords were left in the radioactive solution for at least that time so that fluxes measured could be assumed to be those of the steady-state condition. After 1·5 h in physiological solution connectives still appeared to show normal electrical responses, even though from analyses of total sodium and potassium in the nerve cord it was found that there was a significant increase in sodium associated with a decrease in potassium after this time (L. E. Tucker, unpublished result).

Efflux measurements. For efflux experiments and for electrical recordings nerve

cords were mounted in a Perspex chamber illustrated in Fig. 1. The nerve cord was arranged so that 2 mm of the penultimate connectives lay across the cylindrical compartment (3), and the three compartments were isolated from one other by petroleum-jelly seals. Compartment 1 contained normal physiological solution and compartment 2 contained flowing 483 mM/l mannitol solution. Compartment 3 contained physiological solution flowing from a gravity-feed system at a regulated rate;
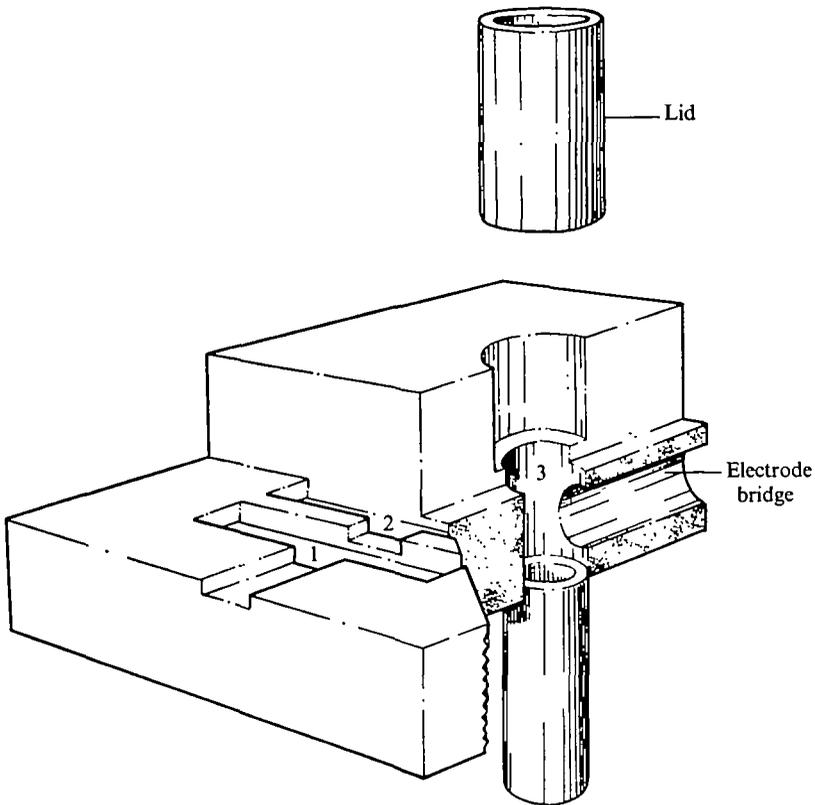

Fig. 1. Diagram of nerve chamber used for extracellular recording of the extraneuronal potential and for ²²Na efflux experiments on the penultimate connectives of the abdominal nerve cord.

the wash-out time for this compartment was found to be less than 2 sec. The effluent from compartment 3 was collected on a moving strip of filter paper for the first half minute of an experiment. Samples over longer time intervals were collected in small bottles and the solution in the bottles was then soaked up with filter paper and dried. All samples were analysed for ²²Na by counting on paper in a PPO/xylene solution in a liquid scintillation counter. At the end of an experiment the radioactive sodium remaining in the portion of the connectives which had been washed was determined by transferring the 2 mm sections of the penultimate connectives to a planchet and counting the activity with a thin end-window G–M tube. Corrections were made for the differences in efficiencies of the liquid scintillation and G–M counting methods.

Electrical recordings. To measure extraneuronal potentials developed upon exposure of the connectives to high-potassium solution, this solution (in which K+ replaced Na+ in the normal solution) was made to flow through compartment 3 in the nerve chamber by the use of a two-way non-return valve which was arranged close to the chamber so as to reduce dead space. Compartment 1 was connected to a high-impedance converter via a saline-agar bridge and compartment 3 was connected to an indifferent electrode via another saline-agar bridge. Continuous recordings of potential changes were made using a Smith Servoscribe potentiometric recorder.

It was found that sodium-free, high-potassium solution slightly increased the rate of sodium efflux, but in intact preparations this effect was extremely small if the exposure time to the high-potassium solution was short, and therefore an estimation of the extraneuronal potential was possible during the course of a ^{22}Na efflux experiment lasting 35 min. However, in de-sheathed preparations the increase in sodium escape with a high-potassium solution was more marked and therefore exposure to the high-potassium solution was only possible after sufficient samples of 'normal' physiological effluent had been collected to enable the slope of the final exponential portion of the efflux curve to be calculated.

RESULTS

^{22}Na efflux and the height of the extraneuronal potential

From a study of the half times for the efflux of ^{22}Na from connectives and height of the extraneuronal potentials developed when connectives were exposed to high-potassium solution, it was evident that there was some correlation between the rate of sodium efflux and the size of the extraneuronal potential developed in the connectives (Fig. 2A, B); the longer the half time for sodium efflux, the larger was the size of the extraneuronal potential.

The time courses of ^{22}Na efflux from two intact pairs of connectives, one which had a large extraneuronal potential (62 mV) and the other with a small one (14 mV), are shown in Fig. 3. From this figure it can be seen that the individual efflux curves are initially complex, but after about 10 min both assume a simple exponential form which is maintained up to 35 min, when the experiments were terminated. Although there is a considerable difference between the two preparations in the rate of escape of sodium during the first 5 min of the experiment, the slopes of the final exponential portions of both curves are very similar. The variation in the initial rate of ^{22}Na efflux is shown clearly in Fig. 4 where the first minute of the efflux from the same two preparations is drawn on an expanded scale. Also included in this figure are efflux curves from two other preparations with extraneuronal potentials of intermediate size.

Graphical analysis. In order to be able to study the first part of the efflux curves more fully, each curve was graphically analysed as shown in Fig. 5.* The final straight

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* It is realized that large errors are inherent in the form of graphical analysis of curves used in this work, and calculation of rate coefficients and unidirectional fluxes are complicated because no single segment of the efflux curve can be identified with a single compartment within the preparation (see Solomon, 1960). Also, the zero-time intercepts of various components do not represent the amount of ^{22}Na in the respective compartments at zero time (Huxley, 1960; Cho *et al.* 1967). Although it is theoretically possible to make mathematical corrections for these errors, it has been considered that a comparison of uncorrected values for different components would be sufficient for the purposes of the present investigation.
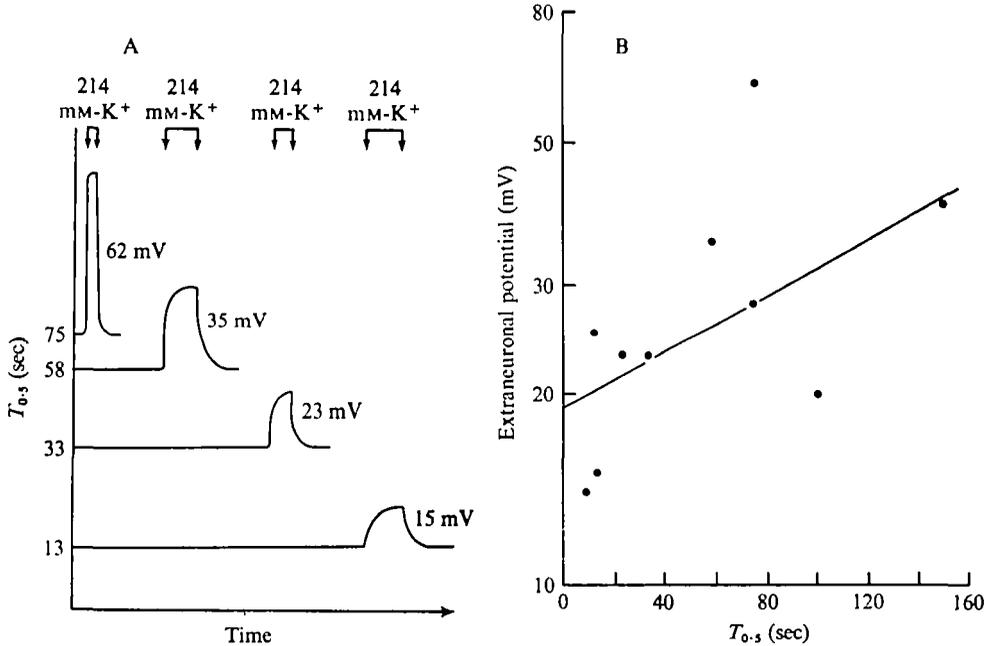
Fig. 2. Relationship between the rate of sodium efflux and the size of the extraneuronal potential developed when connectives were exposed to high-potassium solution. A. Continuous records of potential changes obtained during exposure of four connectives to a high-potassium sodium-free solution. The records are so disposed that the base-line of each record is located on the vertical scale at a level which indicates the $T_{0.5}$ of the preparation from which the record was obtained. The locations of the records on the horizontal scale are arbitrary. B. The size of the extraneuronal potential plotted against $T_{0.5}$ for total efflux. The straight line represents a calculated regression curve.
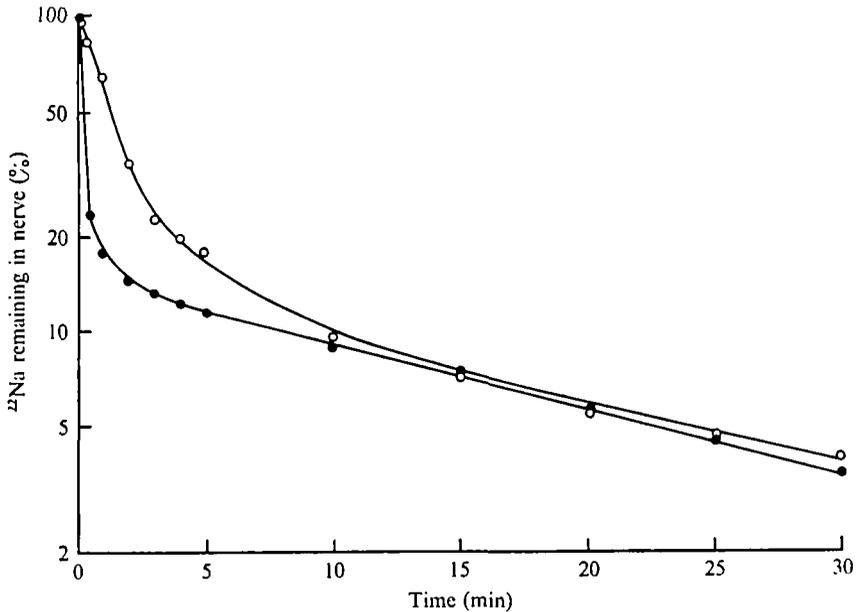

Fig. 3. A semi-logarithmic plot of ²²Na efflux from two preparations, one (open circles) with an extraneuronal potential of 62 mV and the other (closed circles) with one of 14 mV.
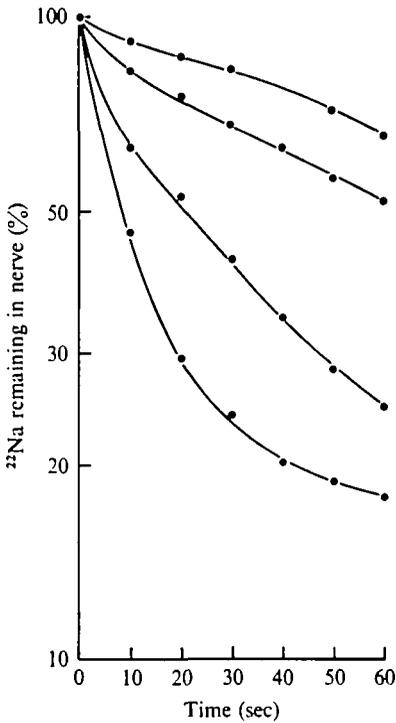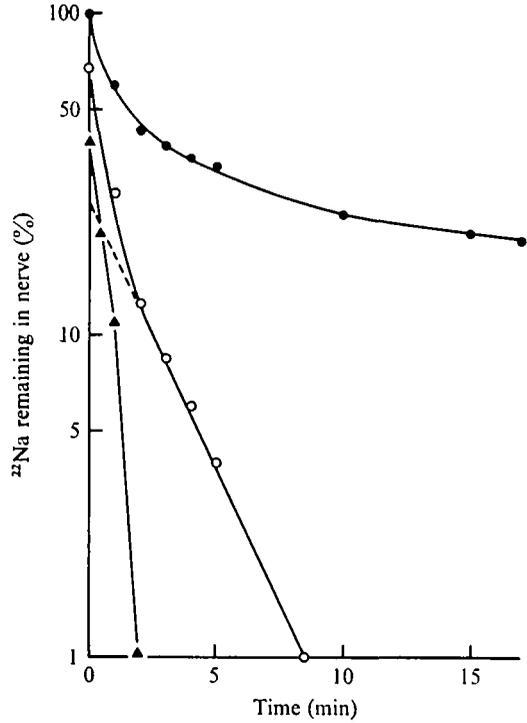
Fig. 4. Fig. 5

Fig. 4. ²²Na efflux from four preparations during the first minute of each experiment.

Fig. 5. Semi-logarithmic plot of ²²Na efflux from a single preparation, illustrating the way in which the efflux curve was graphically analysed into three components. Lines have been fitted by eye. Slow component is the straight-line portion of closed circles curve; intermediate component is the straight-line portion of open circles curve; fast component is represented by triangles.

portion of the curve was extrapolated to zero time and subtracted from the main curve so that a second curve was obtained. This curve, like the total efflux curve, was complex initially, but after about two minutes it assumed a simple exponential form. This curve was also graphically analysed into two components. Therefore, the curve representing the total loss of ²²Na from the connectives may be looked upon as being made up of at least three components with very different time constants; for the preparation shown the slowest component had a half time of 21 min, the intermediate a half time of 114 sec and the fastest component, which is initially complex, a half time of 30 sec. The half times for the slow components did not show much variation from preparation to preparation, the mean value being 16·0 ± 1·4 min. However, the rates of efflux of sodium in the intermediate and fast components were much more variable, the mean values being 102·2 ± 17·7 and 29·7 ± 10·1 sec, respectively.

Relationship between half times of the three components

There appeared to be a trend for preparations which had long half times for the fast component to have long half times for the intermediate component. However, when values of half times of the intermediate component are plotted against those of

the fast components for individual preparations the points are very scattered (Fig. 6). No correlation was found between half times of the fast and slow components or of the intermediate and slow components. This tends to indicate that different factors are limiting the rate of ^{22}Na efflux in the three components.
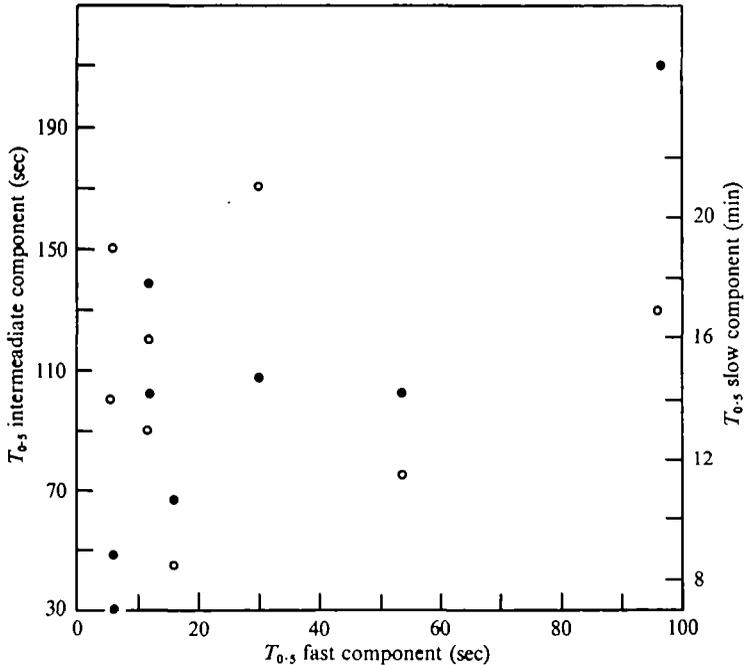

Fig. 6. Comparison of half-times for sodium efflux in the fast, intermediate and slow components. $T_{0.5}$ for the intermediate component (closed circles), and $T_{0.5}$ for the slow component (open circles) have been plotted against the corresponding $T_{0.5}$ of the fast component for each preparation.

Correlation between the extraneuronal potential and the efflux components

Because the fast component was not a simple exponential and therefore the slope varied with time, it was impossible to directly compare slopes of the fast components of different preparations, and the most convenient measure for comparison was found to be the time at which the fast component intercepted the X-axis, an approximate estimate of when 99 % of the sodium in the nerve at the beginning of the experiment had been exchanged. Similarly, the times at which the intermediate and slow components intercepted the X-axis were used to see whether there was any correlation between the rate of sodium efflux in these components and the height of the extra-neuronal potential developed in high-potassium solution. No correlation was found between the rate of sodium efflux in the slow components and the height of the extra-neuronal potential (Fig. 7A), and for the intermediate component there appeared to be a slight correlation (Fig. 7B), although it was not significant ($0.2 > P > 0.1$). It was only with the fast component that a significant correlation ($P < 0.001$) between the rate of sodium efflux and the height of the extraneuronal potential could be found (Fig. 7C).
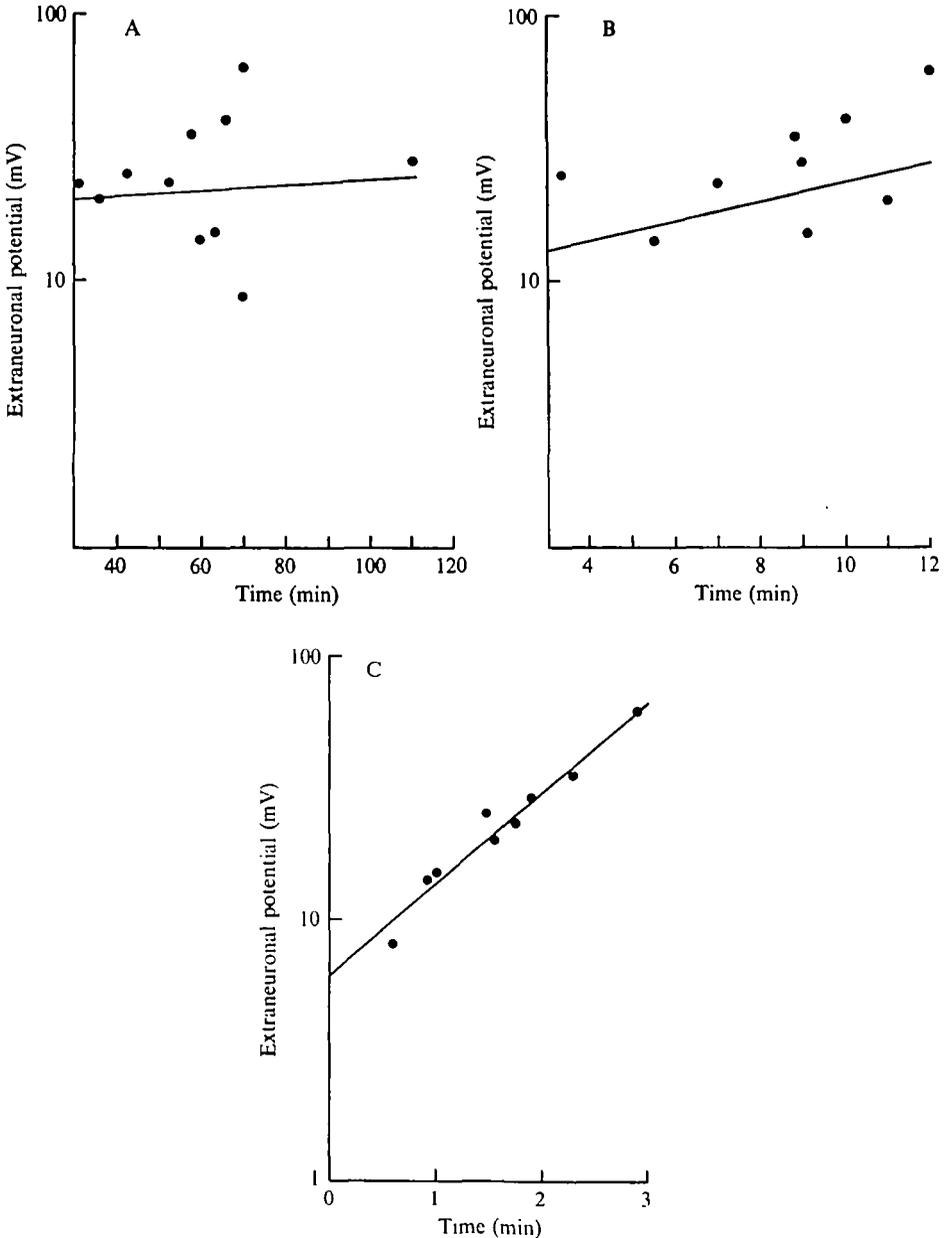
Fig. 7. Relationship between the rate of ¹¹Na efflux in each component and the height of the extraneuronal potential. Values on the time axis represent the time at which 99 % of the sodium in the connective at the beginning of the experiment had been exchanged. On each graph calculated regression lines have been drawn. (Regression lines were calculated as the straight line of best fit for the data points using log₁₀ y values.) A. Slow component. B. Intermediate component. C. Fast component.

Effects of de-sheathing. It was evident from an examination of the total efflux curves that de-sheathing of connectives greatly speeded up the rate of escape of ²²Na (Fig. 8). The effect of de-sheathing on the sodium efflux is summarized in Table 1, where mean

half times for the three components for all intact connectives are compared with those for de-sheathed preparations. A significant increase in ^{22}Na efflux for all components is brought about by de-sheathing and the increase is most pronounced for the fast component.
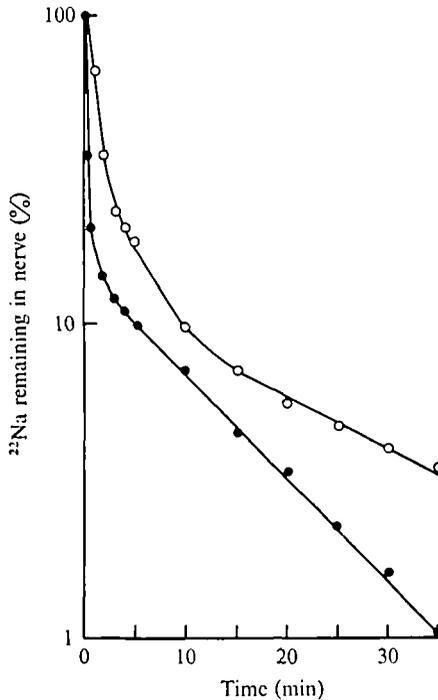

Fig. 8. Efflux of ^{22}Na from intact (open circles) and de-sheathed (closed circles) connectives.

Table 1. *Effect on ^{22}Na efflux of de-sheathing connectives*

	Fast component $T_{0.5}$ (sec)		Intermediate component $T_{0.5}$ (sec)		Slow component $T_{0.5}$ (min)	
Sheathed	29.7 ± 10.1	$\Big\} P < 0.001$	102.2 ± 17.7	$\Big\} P < 0.05$	16.0 ± 1.4	$\Big\} P < 0.02$
De-sheathed	2.0 ± 0.82		33.0 ± 2.72		8.5 ± 0.47	

DISCUSSION

The results outlined in this paper confirm that there is a relatively rapid exchange of sodium between the bathing medium and the nerve cord in *Periplaneta americana* as had been demonstrated earlier by Treherne (1961 *a–d*, 1962 *a*). Our results show, however, as suggested earlier (Pichon & Treherne, 1971), that there is a clear relation between the state of the preparation (indirectly estimated from the size of the extra-neuronal potential) and the unidirectional flux of ^{22}Na ions: the higher the extra-neuronal potential, the slower the exchange. This was to be expected from previous results on the depolarizing and blocking effect of high-potassium solutions on the C.N.S. of the cockroach (Treherne *et al.* 1970; Pichon & Treherne, 1970, 1971; Pichon *et al.* 1971). Since the extraneuronal potential is supposed to originate at the level of the peripheral cellular sheath (perineurium), it might be expected, *a priori*,

that the main restriction to ion movement was situated at this same level. In this case the whole c.n.s. could be considered as a single-compartment system (Fig. 10A), the efflux approximating to a single diffusion process defined by a curve whose slope would be directly related to the permeability of the peripheral barrier (B_{1-0}). Fig. 9 shows that in fact the experimental curve does not fit with a theoretical diffusion curve, the efflux of [22]Na from the preparation being much faster during the first minutes than theoretically expected.
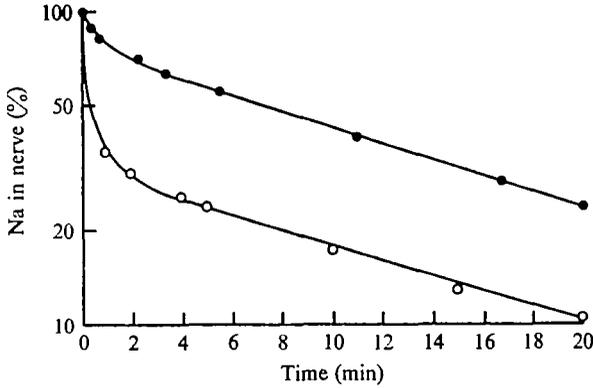
Fig. 9. Theoretical curve (closed circles) for diffusion from a single compartment (calculated from Hill's (1928) equations for diffusion of a substance from a cylinder) and a [22]Na efflux curve obtained for cockroach connectives (open circles).
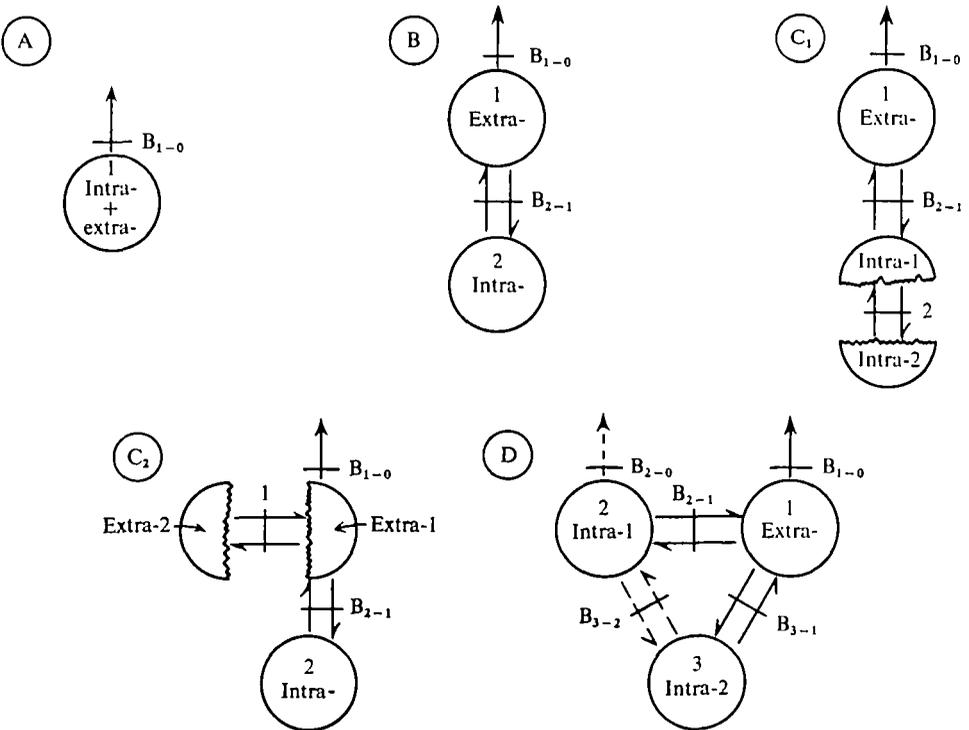

Fig. 10. Possible compartment models for a cockroach connective. A. Single compartment. B. Two homogeneous compartments. C. 1 and 2, two compartments, one of which is non-homogeneous. D. Three compartments.

The time course of the efflux during the first 5 min shows considerable variation between different preparations. This is particularly clear if one considers the first minute, as in Fig. 4, from which it can be seen that preparations showing comparatively large extraneuronal potentials exhibit an approximately first-order exponential loss of radioactivity during the last 50 sec, whereas those with small extraneuronal potentials behave in a more complex manner and lose their radioactivity several times more quickly.

Efflux components

Graphical analyses of the efflux curves have shown that each individual efflux curve could be divided into at least three components. This eliminates the possibility that the system we have been dealing with was a two-compartment system, one extracellular compartment and one cellular compartment with the main restriction between the two (Fig. 10B).

Fast component. It would seem reasonable to assume that at least most of the fast component of the efflux from the cockroach connectives is an extracellular fraction. Some ions which had been adsorbed on the surface of the nerve sheath may also be included in this fast component, although counts for the first 2 sec of the 'washing' period were not included in the total counts. This possibility has been checked by comparing the radioactivity associated with the isolated nerve sheath with the radioactivity of the nervous tissue after extrusion from the connective using a small roller as described by Baker *et al.* (1962) for the squid axon; only 10 % or less of the total radioactivity was associated with the nerve sheath. Furthermore, it is not unlikely that the fast component may, at least partly, have a perineurial-glial origin involving free diffusion or exchange diffusion mechanisms (see Ussing, 1949; Shaw & Stobbart, 1963) across the cell membrane.

In some preparations the fast component appeared to approximate to a single exponential. In most cases, however, it was definitely a more complex curve (sigmoid or humped) which could be interpreted, according to van Liew (1967), as an indication that the curve is a combination of both linear and exponential processes.

The very significant correlation between the rate of efflux of this component and the extraneuronal potential is in fair agreement with the interpretation of the ionic dependence of the extraneuronal potential (Pichon *et al.* 1971). According to the theoretical model system which has been chosen as best representing the experimental behaviour of the extraneuronal potential the effect of high external concentrations of K^+ ions would be to depolarize the outwardly facing perineurial cell membrane, and the departure of the observed potentials from the values predicted by the Nernst equation could be accounted for in terms of the short-circuiting effect due to finite ionic permeability of the tight junctions between perineurial cells. The present experiments show effectively that the faster this fast fraction (i.e. the leakier the peripheral barrier) the smaller the extraneuronal potential. This correlation is not, however, a proof that the only route used by the ions to move out of and into the C.N.S. is via the perineurial clefts and tight junctions. The possibility of an alternative route, possibly intracellular via the glial cells (which have been shown to be linked together by low-resistance pathways (Lane & Treherne, 1969)) and also affected by factors like stretching or drying, cannot be eliminated.

Intermediate component. The intermediate component of the efflux is similar in its temporal characteristics to the slow component demonstrated by Treherne (1961 *d*, 1962 *a*) in the c.n.s. of *Periplaneta americana*. Although no experiments with metabolic inhibitors have yet been carried out in the present investigation, it seems reasonable to assume that, as shown by Treherne (1961 *b*, *c*, 1966), this fraction is affected by metabolic inhibitors, ouabain and potassium-free solution. It can thus be attributed to a cellular fraction, the ^{22}Na movements being limited by the passive and active properties of the cellular membrane (B_{2-1}). The rate of ^{22}Na efflux of this component shows some correlation with the size of the extraneuronal potential ($0.2 > P > 0.1$) which could easily be interpreted by assuming that the same factors that affect the permeability of the tight junctions may also significantly affect the properties of the cellular membrane.

Slow component. The slow component is probably identical to the very slow component described by Treherne (1961 *b*, *c*), but he was unable to study it because of the very low radioactivity left in this region. According to Treherne (1961 *b*) metabolic inhibitors apparently did not affect this fraction after a relatively short exposure. (However, it remains possible that the inhibitors had not had the necessary time to reach their site of action, as demonstrated for the squid axon poisoned with cyanide (Baker *et al.* 1969).) This fraction, like the intermediate one, may be cellular, for other excitable tissues exhibit two intracellular sodium fractions which can be separated by a variety of means, such as differential sensitivity to ions and metabolic inhibitors (Baker *et al.* 1969), electrical stimulation (Keesey & Walgren, 1965; Carlson & Treherne, 1969) or by nuclear magnetic resonance studies (Cope, 1967 *a*, *b*). These two different fractions may originate from the non-homogeneous cellular compartments (Fig. 10 C) or from the existence of two different kinds of cells (Fig. 10 D). In the latter case the intermediate component might represent the intracellular fraction from the glial cells and the small axons, the slow one originating from the giant axons which are isolated from the extracellular space by the entire length of the mesaxon channels. This last possibility seems the most likely at this stage.

This component is clearly not related to the extraneuronal potential production ($P \sim 0.8$).

The relative proportions of ^{22}Na in the three efflux components were essentially the same in all the preparations except those subjected to maximum stretching. They were respectively, 45 % in the fast fraction, 40 % in the intermediate and 15 % in the slow one. Although absolute values would be necessary to calculate the amount of sodium contained in each fraction, it can be seen that the extracellular component, which represents only 10–20 % of the total connective volume (Treherne, 1961 *d*, 1962 *a*), must be comparatively rich in Na$^+$ ions. The relative constancy of the sizes of the three fractions, despite variation in the restriction between compartments 2 and 1, and compartment 1 and the external solution (see Fig. 10 D), argues in favour of a stability in the size and ionic content of the three compartments. When the connectives were much stretched, the modifications were more important and were similar to those which appear on de-sheathing.

Effects of de-sheathing

This effect is very dramatic as could be expected from electrophysiological and ultrastructural results. It is somewhat similar to that observed in vertebrate nerve (Dainty & Krnjević, 1955; Shanes, 1954) but is in contrast with the finding of Treherne (1961 c, d) that the rates of sodium efflux were essentially the same in intact or partially de-sheathed ganglia of *Periplaneta*. The most likely explanation for this apparent discrepancy is that an 'intact' ganglion is much more leaky than an 'intact' connective because all the segmental nerves leading from the ganglion have to be cut during the dissection. Furthermore, the ganglia were most often only partially de-sheathed in Treherne's experiments, whereas the connectives were completely de-sheathed in the present experiments.

In parallel with the absence of extraneuronal potentials the sodium efflux in de-sheathed connectives is speeded up by a factor of five (according to Fig. 9), all three fractions being affected and their relative sodium content modified from the above figures of 45, 40 and 15 % for the fast, intermediate and slow fractions respectively, to 49, 45 and 6 %. The changes in the kinetics of these three fractions are significant and are more marked in the fast component (mean increase 15 times) than in the intermediate (about three times) of the slow (less than twice). One is aware that, as for the other preparations, a better comparison would have been obtained from absolute values, for the total amount of sodium in the stretched and de-sheathed connectives may have been different from the 'normal' sheathed ones. The sodium content of the de-sheathed connectives after the same equilibration period is likely to be higher than in intact preparations, so that a relative speeding up on de-sheathing would correspond to an even larger absolute change in the rate of sodium efflux. Ultrastructural studies have shown that de-sheathing could destroy the tight junctions at the inner ends of the perineurials clefts (Lane & Treherne, 1970) and isolated sheaths have been seen to consist of the neural lamella with some patches of perineurium adhering to it (N. J. Lane, unpublished observation). It is very likely, therefore, that the properties of the membrane of the perineurial and adjacent glial cells are much affected by de-sheathing, as they become permeable to molecules as large as peroxidase (Lane & Treherne, 1970). These modifications of the peripheral barrier can account for the speeding up of the fast fraction of the efflux curve. The modifications of the kinetics of the intermediate fraction can have the same origin as those already observed in sheathed preparations showing small extraneuronal potentials (see p. 11) and thus correspond to a modification of the membrane properties of the cells together with the absence of restriction in the extracellular channels which are supposed to be opened up by de-sheathing. The interpretation of the speeding up of the slow fraction could be rather simple and imply a simple increase of the permeability of the internal barrier (B_{3-1}) due to mechanical strain during de-sheathing. If one assumes the slow fraction to originate in the giant axons, this barrier (B_{3-1}) (Fig. 10 D) is basically complex and can be divided into two components: (1) the restriction due to the Schwann cell layer (cf. Treherne *et al.* 1970) and (2) the restriction due to the properties of the axonal membrane itself. It can be assumed that, in intact preparations, the strongest restriction is that due to the mesaxon channels; these have been seen to be rather tight and to be filled with electron-dense material which, according

to Ashhurst (1961), might be mucopolysaccharides which will slow-down the movement of ions from the extraaxonal fluid to the extracellular fluid (Lane & Treherne, 1970). The restriction due to the giant axonal membrane might be similar to that found for the other nerve cells (cf. intermediate component) and would, in these conditions, have no effect on the overall kinetics of the slow component. The speeding up of the slow fraction, on de-sheathing, must accordingly originate from an enlargement of the mesaxon channels, as is shown in electron microscope pictures, of highly stretched connectives (Lane & Treherne, 1970), and not from a modification of the properties of the axonal membrane (which could occur at the same time).

On the other hand, if the slow fraction corresponds to an intracellular and/or extracellular sodium store (model C), one has to assume that the removal of the sheath alters the rate of binding of Na^+ ions to the sites in this store. This could result, in the case of an extracellular store (model C2), from an enlargement of the extracellular space. It is not easy to interpret the case of an intracellular store.

The changes in the relative sizes of the different fractions are very difficult to interpret in the absence of absolute values. If the slow component does originate in the giant axons (Fig. 10D), these figures would seem to indicate that deliberate stretching or de-sheathing increases the rate of diffusion from a part of the fraction, for instance by breaking down some of the restriction in the mesaxon, as mentioned above. However, if one takes into account the fact that the de-sheathed preparations were loaded *in vitro* after de-sheathing, another tentative explanation can be proposed. It has been seen (see above, p. 442) that isolated nerve cords exposed for 1 or 2 h to the saline we used (214 mM/l Na) in these experiments significantly gained sodium and lost potassium even though they were ligatured at the two ends. From the above results on the effect of stretching and de-sheathing it seems reasonable to assume that this effect is more important for de-sheathed preparations than for intact ones, the increase in total sodium corresponding mainly to an increase in the amount of sodium in the two more external compartments, that is, the extracellular compartment and the 'glial-small axon' compartment. In that case the efflux would consist of two large (fast and intermediate) fractions followed by a comparatively small (slow) fraction, although the total amount of Na^+ in this last fraction would be the same as in the intact preparations, or even higher.

Before trying to briefly correlate our results with those of electrophysiological experiments, it seems important to emphasize that the above interpretations of the experimental results are speculative and must be considered only as an attempt to analyse the properties of a relatively simple nerve preparation. Much more needs to be known about the structure and the behaviour of the system in various experimental conditions before definite conclusions can be drawn. It has been demonstrated, for instance, that the cytoplasm of various glial processes within the nerve appears to be linked by numerous tight junctions between adjacent glial membranes (Lane & Treherne, 1969). They may represent low-resistance pathways as in the leech (Kuffler & Potter, 1964) and allow relatively free movement of ions to occur between cells. The relative importance of this glial pathway (shown in dotted lines, Fig. 10 D) is not known. It may play a particularly important role in de-sheathed preparations where it has been seen (Lane & Treherne, 1969, 1970) that reaction product from peroxidase is found in the perineurial cells and within the cytoplasm of the glial cells

adjacent to a de-sheathed area of cockroach ganglia, and that peroxidase molecules are able to move between adjacent glial elements of the mesaxon via tight junctions (Lane & Treherne, 1969; Treherne *et al.* 1970).

The effects on the kinetics of sodium efflux of stretching or de-sheathing the connectives are very clearly those which one would have expected from electro-physiological experiments (Twarog & Roeder, 1956; Treherne, 1962*b*; Pichon & Boistel, 1965; Treherne *et al.* 1970; Pichon & Treherne, 1970; Pichon *et al.* 1971). The fact that the present experiments were carried out in steady-state conditions, whereas the electrophysiological ones were carried out in non-steady-state conditions (excess of K^+ or lack of Na^+ in the bathing medium) is of importance if one wants to compare the two kinds of results. In non-steady-state conditions electrochemical gradients are created which may strongly modify the permeability characteristics of the system, especially at the periphery of the connectives where these gradients may be large. It has been previously demonstrated that the perineurial barrier behaved electrically more or less like a membrane but that there was a considerable departure between the observed extraneuronal potentials and the values predicted from the Nernst equation. This has been interpreted as the results of the short-circuiting effect due to the finite permeability of the tight junctions (Pichon *et al.* 1971). It was also found, however, that the size of the extraneuronal potential depended upon the physiological state of the preparation (Pichon & Treherne, 1970). Some recent values obtained in the present experiments and by others (P. K. Schofield & J. E. Treherne, unpublished) in intact preparations were much closer to those predicted from the Nernst equation. This leads us to think that *in vivo* the 'leak' might be much reduced and the net flux of potassium at the 'balanced' state (cf. Conway, 1957) minimal; an increase in the external potassium concentration in these conditions would result in only a small increase in the extracellular potassium concentration. A similar phenomenon might also account for the apparent impermeability of the peripheral barrier to sodium ions in sodium-free media (see Treherne & Pichon, 1971). This purely passive system might be linked to an active regulation by the perineurial or glial cells.

CONCLUSION

The conclusion which can be drawn from these results is that different barriers situated at different levels of the c.n.s., and not a single barrier, regulate the movement of ions in steady-state conditions. Various mechanical treatments can modify the effectiveness of these barriers, the first to be affected being the peripheral one (the 'blood-brain barrier') which in the intact animal may well be the most important during variations in the ionic composition of the blood.

SUMMARY

1. It has been found that the efflux of sodium from intact penultimate connectives of the ventral nerve cord of the cockroach, *Periplaneta americana*, can be represented by three components with half times of approximately 30 sec, 100 sec and 16 min.

2. The rate at which sodium escapes in the fast component is correlated with the height of the extraneuronal potential developed when a connective is exposed to a

high-potassium bathing medium, the slower the rate of sodium escape the larger being the size of the extraneuronal potential. This gives support to the hypothesis that the extraneuronal potential is a diffusion potential brought about by restriction to ion movement imposed by the tight junctions at the inner ends of the perineurial clefts.

3. De-sheathing a connective greatly speeds up the rate of sodium efflux, the fast component being the most affected.

4. These results can be explained in terms of a three-compartment system, the properties of which are affected by stretching and de-sheathing.

5. It is concluded that the movement of ions is regulated at different levels of the c.n.s. but that the peripheral barrier (which is responsible for the production of the extraneuronal potential) may be the most important one in non-steady-state conditions.

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