EXTRANEURONAL POTENTIALS AND POTASSIUM DEPOLARIZATION IN COCKROACH GIANT AXONS

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INTRODUCTION

Recent investigations to test the effects of high potassium concentrations on the membrane potentials of cockroach giant axons in intact connectives have indicated that the observed variations in potential could not be related to a simple depolarization of these axons (Pichon & Treherne, 1970; Treherne, Lane, Moreton & Pichon, 1970). This conclusion is based on the observation that the apparent depolarization of impaled giant axons is not associated with any significant reduction in the amplitude of the action potentials and that essentially similar potential changes could be recorded with the micro-electrode tip located in an extracellular position. The above results clearly suggest that there is a restriction to the intercellular diffusion of potassium from the bathing medium. This restriction is unlikely to be associated with the superficial connective tissue sheath which is relatively permeable to radioactive ions (cf. Treherne, 1961, 1962; Eldefrawi & O'Brien, 1967) and to molecules as large as those of peroxidase (Lane & Treherne, 1969, 1970). On the basis of the observed restriction of the latter molecule at the inner end of the intercellular cleft which traverses the perineurium it was suggested that the occlusion represented by septate desmosomes and tight junctions in this region (Maddrell & Treherne, 1967) could also restrict the penetration of small water-soluble cations into the underlying extracellular system (Pichon & Treherne, 1970; Treherne *et al.* 1970). It was further tentatively suggested that the rapid positivation of the micro-electrode tip, in intact preparations bathed with high-potassium solutions, could result from the more rapid penetration of potassium ions relative to the outward movement of sodium ions from the extracellular system.

There was considerable individual variation in the extent of the extraneuronal potential change resulting from elevation of the external potassium concentration in intact connectives (Pichon & Treherne, 1970; Treherne *et al.* 1970). It was also observed that there was an apparent inverse relationship between the magnitude of this potential change and the rate of depolarization of the giant axons in these preparations. The potential was minimal in preparations mounted under tension, being maximal in unstretched preparations. It appears, therefore, that both the accessibility of the giant axons to external potassium ions and the magnitude of the extraneuronal positivation resulting from an increase in the external concentration of this cation depend in a critical way upon the state of the individual preparations. This factor is

also of considerable relevance in the interpretation of the results of radioisotope experiments (cf. Treherne, 1961, 1962; Eldefrawi & O'Brien, 1967) in which the nerve cords were subjected to varying degrees of experimental insult. The present investigation was, therefore, initiated in an attempt to elucidate the nature of the extra-neuronal potential changes produced with elevated external potassium concentrations, and, in particular, to determine the effect of various experimental treatments on this potential change.

METHODS

Micro-electrode experiments were carried out using the second nerve chamber described in an earlier publication (Treherne et al. 1970). With this apparatus the penultimate abdominal connective was supported on a piece of foam rubber to facilitate penetration by the micro-electrode without appreciable application of tension to the nerve cord. The giant axons were stimulated, by silver-wire electrodes, from the terminal connective by a series of rectangular current pulses (0·5 sec^{-1}, 0·2 msec duration) at low output impedance via an R.F. isolating unit. The glass micro-electrodes were filled with 3·0 M-KCl and had resistances of between 10 and 30 MΩ and tip potentials of less than 5·0 mV. They were used in conjunction with a high-impedance F.E.T. input stage (Bio-electric NFI), in most cases adjusted to a gain of 2, coupled to a Tektronix 561 oscilloscope. Continuous d.c. recordings were made using a Smith Servoscribe potentiometric recorder.

In other experiments the 'sucrose-gap' technique (Stämpfli, 1954) was employed. With this technique the nerve chamber used consisted essentially of three parallel compartments isolated from each other by petroleum-jelly seals (Fig. 1). The central compartment contained isotonic sucrose or mannitol (483·0 mM/l) solutions. The latter solution was preferred to sucrose because this substance had a lower molecular weight and, as it is produced synthetically, is likely to contain less contamination with ionic materials. The right-hand compartment contained the experimental solution flowing at a regulated rate. It was connected to the high-impedance amplifier by a saline-filled agar bridge. The left-hand compartment was filled with normal saline or isotonic KCl solution and was connected to the indifferent electrode via a second saline–agar bridge. The arrangement enabled the intracellular potentials of the giant axons in the right-hand compartment to be measured with an attentuation of about one-third (Pichon, unpublished results).

With both recording systems perfusion of the nerve chamber was achieved using a gravity-fed system, at a constant rate of 5–10 ml/min from a series of elevated reservoirs. As in a previous investigation (Treherne et al. 1970) rapid changes of solution, with minimal mixing, was carried out using a multiway non-return valve.

Varying degrees of tension were applied to the isolated nerve cord, using the sucrose-gap chamber, by two laterally positioned Prior micromanipulators, the ligatured ends of the nerve cords being connected to the micromanipulators by cotton threads. To test the effects of drying the penultimate connective the chamber containing the experimental solution was emptied and a piece of filter paper was placed against the side of the chamber (to avoid direct contact with the exposed length of the connective). In some experiments the penultimate connective was de-sheathed using sharpened steel needles.

The normal physiological solution employed in these experiments was that devised by Yamasaki & Narahashi (1959): $210 \cdot 0$ mM/l Na$^+$, $3 \cdot 1$ mM/l K$^+$, $1 \cdot 8$ mM/l Ca^{2+}, $216 \cdot 9$ mM/l Cl$^-$, $0 \cdot 2$ mM/l H$_2$PO$_4^-$ and $1 \cdot 8$ mM/l H$_2$PO$_4^{-2}$. In the high-potassium solution the sodium ions were replaced by potassium ions.
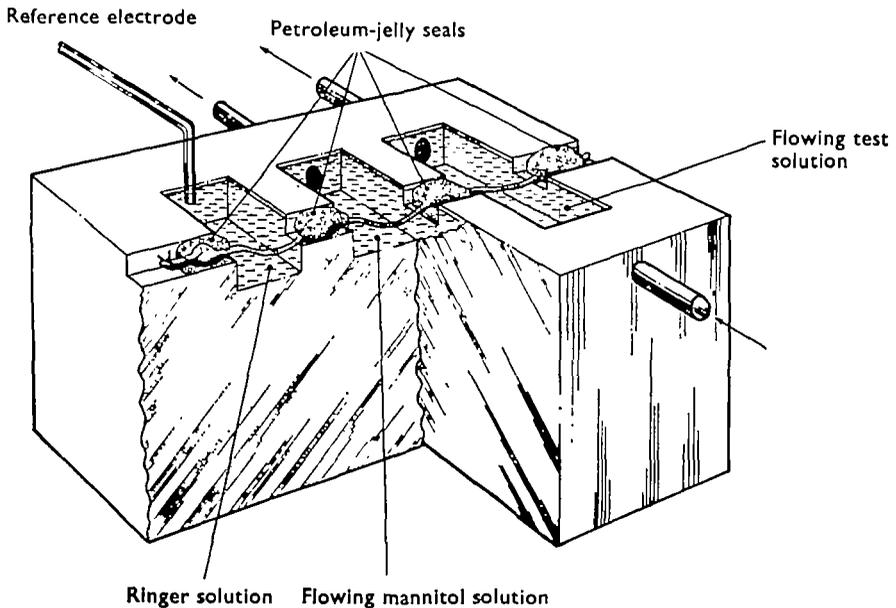

Fig. 1. Diagram of the experimental chart used with the sucrose-gap technique. The recording electrode was located in the drainage line of the right-hand compartment.

RESULTS

Micro-electrode experiments

Fig. 2 illustrates the differences in the electrical responses of giant axons which were obtained with three preparations following exposure of the connectives to the same high-potassium solution ($214 \cdot 0$ mM/l K$^+$). Fig. 2A shows a rapid apparent depolarization which is not associated with any equivalent reduction of the action potentials in an intact connective. Fig. 2B illustrates the alternative response obtained with intact connectives, that is, a depolarization which is associated with a decline in the amplitude of the intracellularly recorded action potentials. Fig. 2C shows the very rapid depolarization obtained with a de-sheathed preparation which is again associated with an equivalent reduction of the recorded action potentials.

As shown in a previous investigation, the rapid positivation measured within giant axons in intact connectives (Fig. 2A) can also be recorded with the micro-electrode tip in an extracellular position and does not represent a true depolarization of the giant axons (Pichon & Treherne, 1970; Treherne *et al.* 1970).

Fig. 3 represents an equivalent experiment performed with an intact preparation showing a minimal extraneuronal potential change and a depolarization of the giant axons following exposure to a high-potassium solution (i.e. corresponding to the situation illustrated in Fig. 2B). It will be seen that there was no appreciable rapid
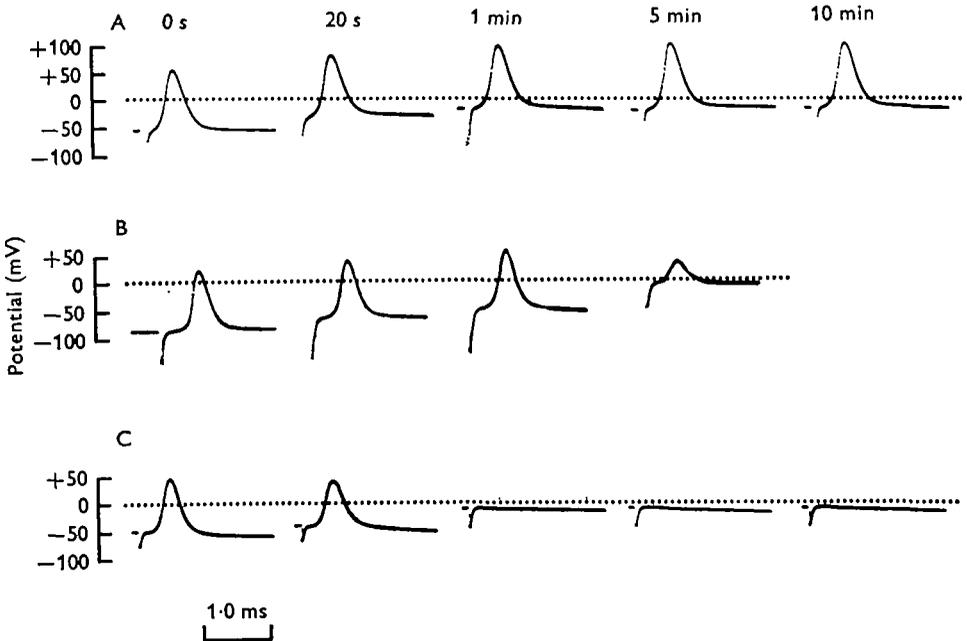
Fig. 2. Illustrating the form of electrical response to high-potassium solution (214 m/Ml), measured with intracellularly located micro-electrodes, in giant axons of penultimate abdominal connectives. A. An intact connective showing a pronounced positivation of the electrode tip with no appreciable reduction of the recorded action potentials. B. An intact connective showing a smaller positivation with reduction in the amplitude of the action potentials. C. A de-sheathed connective showing both the rapid reduction in resting potential and subsequent conduction block.
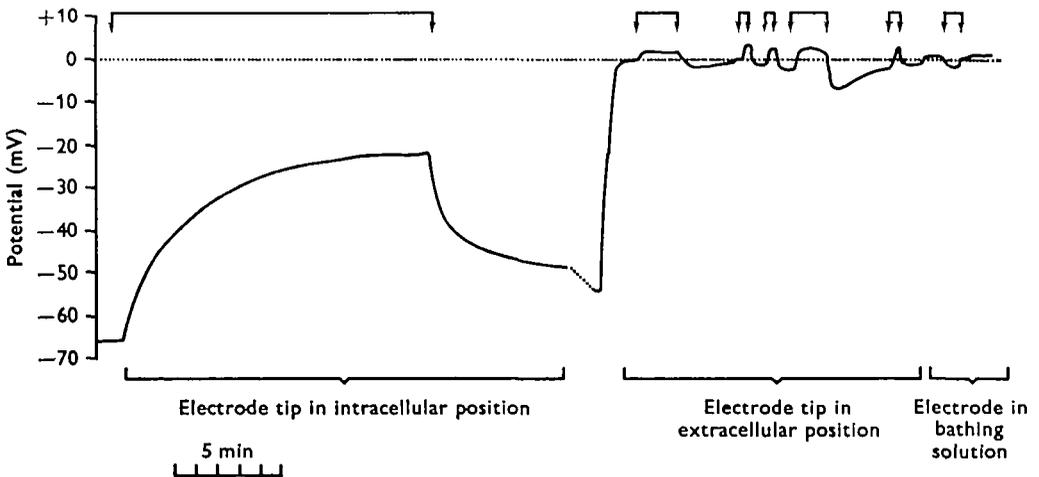

Fig. 3. Potential changes in an intact connective in response to increase in the potassium concentration (214·0 mM/l) measured with a micro-electrode. The intracellularly recorded potential showed a continuous and relatively slow decline. With the electrode tip in an extracellular position there was only a small and unstable positivation, in response to elevation of the external potassium concentration. The fourth exposure to high-potassium solution produced transient reversal of potential on return to normal solution. The effect of the high-potassium solution with the electrode tip in the bathing is also shown. The continuous lines with arrows represent periods of exposure to the high-potassium solution.

positivation of the intracellularly located electrode tip, but only a continuous and relatively slow reduction in the recorded potential. Of particular interest are the potential changes measured in this preparation, following exposure to both high-potassium and normal solutions, with the electrode tip located in an extracellular position. It will be seen from Fig. 3 that there was only a relatively small positivation, of 5–6 mV, following exposure to high potassium in the bathing medium. Further-more, it was found that the recorded potentials were not stable but tended towards a mean value in both high-potassium and normal solutions. This effect is particularly apparent following the final exposure to high-potassium solution, when return to normal solution was associated with a marked and transient reversal of potential.
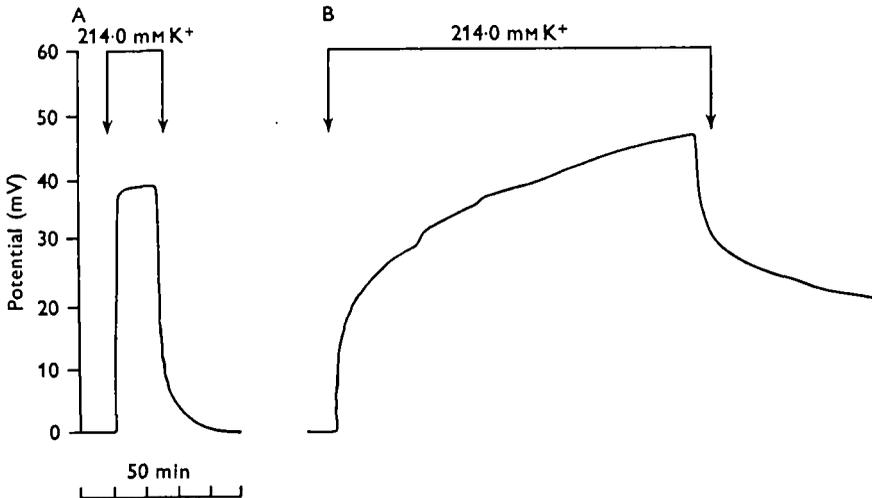

Fig. 4. Potential changes, measured using the sucrose-gap technique with intact connectives, resulting from elevation of the potassium concentration of the bathing medium (to 214 mM/l). A. Shows the rapid potential change corresponding to the positivation illustrated in Fig. 1A. B. Illustrates the relatively slow and continuous potential change observed in some prepara-tions.

Sucrose-gap experiments

This technique enables external recordings to be made of d.c. potential changes and possesses the obvious advantage that such changes can be recorded without the necessity of penetration by micro-electrodes, a procedure which could be envisaged as causing an artificial leakage of ions between the extracellular system and the bathing medium. As will be seen from Fig. 4, both extremes of potential changes associated with elevation of the external potassium concentrations were also recorded using this technique. The fact that the relatively slow and continuous potential change was obtained using the sucrose-gap technique (Fig. 4) argues against the possibility that this type of potential change recorded intracellularly resulted largely from micro-electrode damage to the tissues.

The results of a previous investigation indicated that the form of the electrical response to increased potassium concentration of the bathing medium depended upon the method by which intact connectives were mounted in the nerve chamber (Treherne *et al.* 1970). These observations have been followed up in this investigation,

using the sucrose-gap technique, by determining the effect of applied tension on the potential change induced by a high-potassium solution. Fig. 5 A illustrates the results of such an experiment. It will be seen that the rapid initial positivation, obtained in the unstretched preparation, was abolished in a nerve cord which had been stretched to 1½ times its original length, the potential change in the latter conditions corresponding to the slower and continuous form associated with the direct depolarization of the giant axons. The time course of the potential change in the stretched connective was, however, considerably slower than that in a stretched de-sheathed connective, in which the observed potential change can be also attributed to direct depolarization of the giant axons (Fig. 5 B).
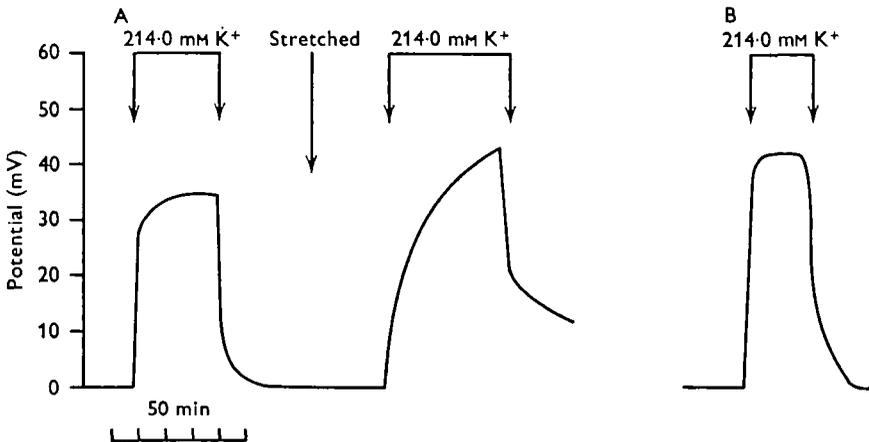

Fig. 5. A. The effect of stretching an intact connective (approximately to 1½ times its original length) on the rapid potential change produced by high external potassium concentration (214·0 mM/l). B. The potential change recorded in a de-sheathed and stretched connective. Both measurements were made using the sucrose-gap technique.
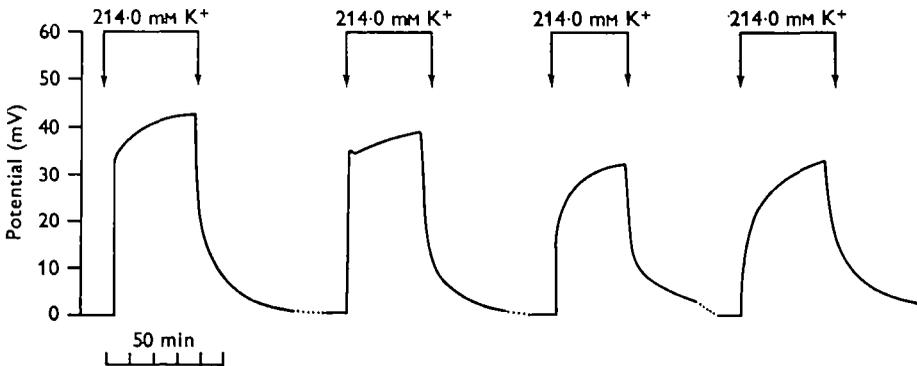

Fig. 6. The effect of drying intact connectives on the rapid potential change produced by high-potassium solution (214·0 mM/l), measured using the sucrose-gap technique. The connectives were exposed to air for successive 1 min periods, following the initial exposure to the high-potassium solutions.

The present results are also of particular interest for their relation to those obtained in previous investigation on the exchanges of radioactive ions in the nerve cord of this insect (Treherne, 1961, 1962). In these investigations the efflux of radioactive ions was

measured in isolated nerve cords and connectives from which superficial radio-activity was removed by placing the preparations on pieces of filter paper, and it seemed possible that such a procedure could somehow alter the properties of the extraneuronal tissue. Fig. 6 shows the effects of draining the compartment containing the experimental solution; the length of connective was then left in contact with air for successive 1 min periods between exposure to normal and high-potassium solutions. It will be seen that this relatively gentle treatment, which did not involve direct contact of the surface of the connective with the filter paper (as in radioisotope experiments) resulted in a progressive change in the form of the electrical response to pulses of high-potassium solution. With each exposure to high-potassium solution the initial rapid potential change was reduced so as to lead finally to the slow and continuous change characteristic of the direct depolarization of the giant axons in intact connectives.

DISCUSSION

A significant feature of these results is the essential similarity between the electrical responses obtained to high external potassium concentrations, using micro-electrodes and using the sucrose-gap technique. This is particularly relevant in the case of the relatively slow potential changes produced by high-potassium solutions in intact preparations. The present results, together with those presented in previous investigations (Pichon & Treherne, 1970; Treherne *et al.* 1970) have indicated that this form of potential change represents a true depolarization of the giant axons, for they are associated with a reduction of the resting potential and eventual blockage of the conducted spike. In this respect this form of potential change is similar to that observed in de-sheathed preparations, but differs very markedly in the much slower time course of the depolarization. The fact that essentially, similar depolarizations were obtained using the sucrose-gap techniques and the micro-electrode techniques would seem to eliminate the possibility that the access of potassium ions to the axon surfaces (observed in preparations in the absence of appreciable extra-neuronal positivations) was facilitated by tissue damage caused by microelectrode impalement. The present investigation has also shown that the extracellular potential changes associated with the relatively slow depolarization of the giant axons observed in intact preparations are of small amplitude. The small extracellular positivation (5–6 mV) observed following exposure to high external potassium concentration was unstable, tending towards a mean value in both high-potassium and normal solutions. Furthermore, the change to normal from high-potassium solution was in that case associated with a transient reversal of potential. These observations accord with the hypothesis advanced in an earlier publication (Treherne *et al.* 1970) that the relatively slow depolarization corresponded to an intercellular diffusion of potassium ions to the axon surfaces, the rate of movement of the cations from the bathing medium being largely determined by the extended diffusion channel represented by the mesaxon cleft and the overlying extracellular system.

The appreciable extraneuronal positivation exhibited by some intact preparations in the presence of high external potassium concentrations has been shown to be associated with a restriction to intercellular ion movements, most probably as a result of occlusions to intercellular clefts at the inner margin of the perineurium (Treherne

et al. 1970; Pichon & Treherne, 1970). The present investigation has shed some light on the nature of the experimental conditions which determine this type of electrical response. In preparations showing maximal extraneuronal potentials variation in response to elevated potassium concentrations, application of tension resulted in change to the relatively slow potential variation associated with depolarization of the axons. A similar effect was obtained by brief exposure of the connectives to air. It follows from the above considerations that both of these treatments must be associated with an increased access of potassium ions to the axon surfaces from the bathing medium, presumably as a result of alteration to the intercellular occlusions at the inner margin of the perineurium (cf. Treherne *et al.* 1970). It is clear, therefore, that the experimental treatment is likely to have a profound effect upon the access of small water-soluble ions into the subperineural extracellular system. This is particularly relevant in the case of some previous radioisotope experiments in which extraneous radioactive solution was removed by gently drying the ganglia and connectives with filter paper. Experiments are at present in progress in this laboratory to test the above effects on the exchanges of radioactive ions in cockroach central nervous tissues.

The present investigation has not elucidated the precise nature of the extraneuronal potential changes resulting from increased potassium concentrations observed in intact preparations. Such an effect could, therefore, result from an extracellular diffusion potential created by the more rapid penetration of potassium ions relative to the outward movement of sodium ions into the bathing medium (Treherne, *et al.* 1970; Pichon & Treherne, 1970). In this case it would seem to be of prime importance to determine, using modified ionic solutions, what is the relative selectivity of this channel to ions other than those of sodium and potassium. Alternatively, it could be envisaged that this phenomenon could result merely from the depolarization of the outwardly facing perineural membrane, depolarization of the inwardly directed one being substantially reduced by the presence of the perineurial tight junctions which would restrict the access of potassium ions into the underlying extracellular channels. It is hoped that current research will help to give some indication of the relative importance of these two factors.

SUMMARY

1. Elevation of the external potassium concentration resulted in a marked positivation of both intracellularly and extracellularly located micro-electrode tips, in intact connectives, with no appreciable reduction in the amplitude of the recorded action potentials.

2. Sucrose-gap experiments showed that this effect was abolished when tension was applied to the nerve and/or if the connectives were briefly exposed to air. In such preparations continuous and relatively slow potential changes were observed, corresponding to depolarization of the giant axons, with only small extracellular positivations of around 5–6 mV.

3. It is concluded that this effect resulted from the access of potassium ions into the extracellular system most probably as a consequence of the disruption of intercellular occlusions at the inner margin of the perineurium, a situation which contrasts with the restricted intercellular penetration associated with preparations exhibiting extracellular positivation. The possible ionic bases of the latter phenomenon are discussed.

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