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A QUANTITATIVE STUDY OF POTASSIUM MOVEMENTS IN THE CENTRAL NERVOUS SYSTEM OF *PERIPLANETA AMERICANA*

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INTRODUCTION

Our understanding of the ionic exchange taking place in insect central nervous tissues is largely derived from radioisotope experiments carried out on isolated connectives or abdominal nerve cords of the cockroach (cf. Treherne, 1961 *a*, *b*, 1962; Eldefrawi & O'Brien, 1967). These studies have indicated that the exchange of small, water-soluble inorganic ions takes place relatively rapidly across the superficial connective tissue sheath and can be represented as an essentially two-stage process. As with some other central nervous tissues (e.g. Ames & Nesbett, 1966; Abbott, 1970) the initial rapidly exchanging fraction was tentatively identified as an extracellular one, the secondary, ouabain-sensitive fraction being identified as the intracellular ion fraction (Treherne, 1962). However, the gross exchanges of inorganic ions between the blood, or bathing medium, and the complicated series of compartments represented by the neurones, glial elements and the complex extracellular spaces are extremely difficult to interpret in terms of the diffusion processes involved in the movements of ions to the extra-axonal fluid. In particular, the role of the narrow and tortuous intercellular channels formed by the closely applied glial and neuronal membranes and the extended mesaxon clefts remains obscure. For these reasons the present investigation was undertaken in an attempt to elucidate the processes involved in the movements of inorganic ions from the blood or bathing medium to the extra-neuronal fluid. The available evidence indicates that cockroach giant axons in de-sheathed preparations respond to changes in the external potassium concentration in a predictable manner (cf. Yamasaki & Narahashi, 1959), similar potential changes also being observed in intact preparations (Twarog & Roeder, 1956; Pichon & Boistel, 1965 *a*, *b*). This cation was therefore chosen for the present study which attempts to link ultrastuctural observations with those made using electrophysiological techniques.

MATERIAL AND METHODS

The material used in this study was the penultimate connectives in the abdominal nerve cord of the cockroach, *Periplaneta americana*. For ultrastructural studies the last two abdominal ganglia and their associated connectives were quickly removed from the animal and fixed, at 4 °C or at room temperature, in one of the following two solutions (p. 111):

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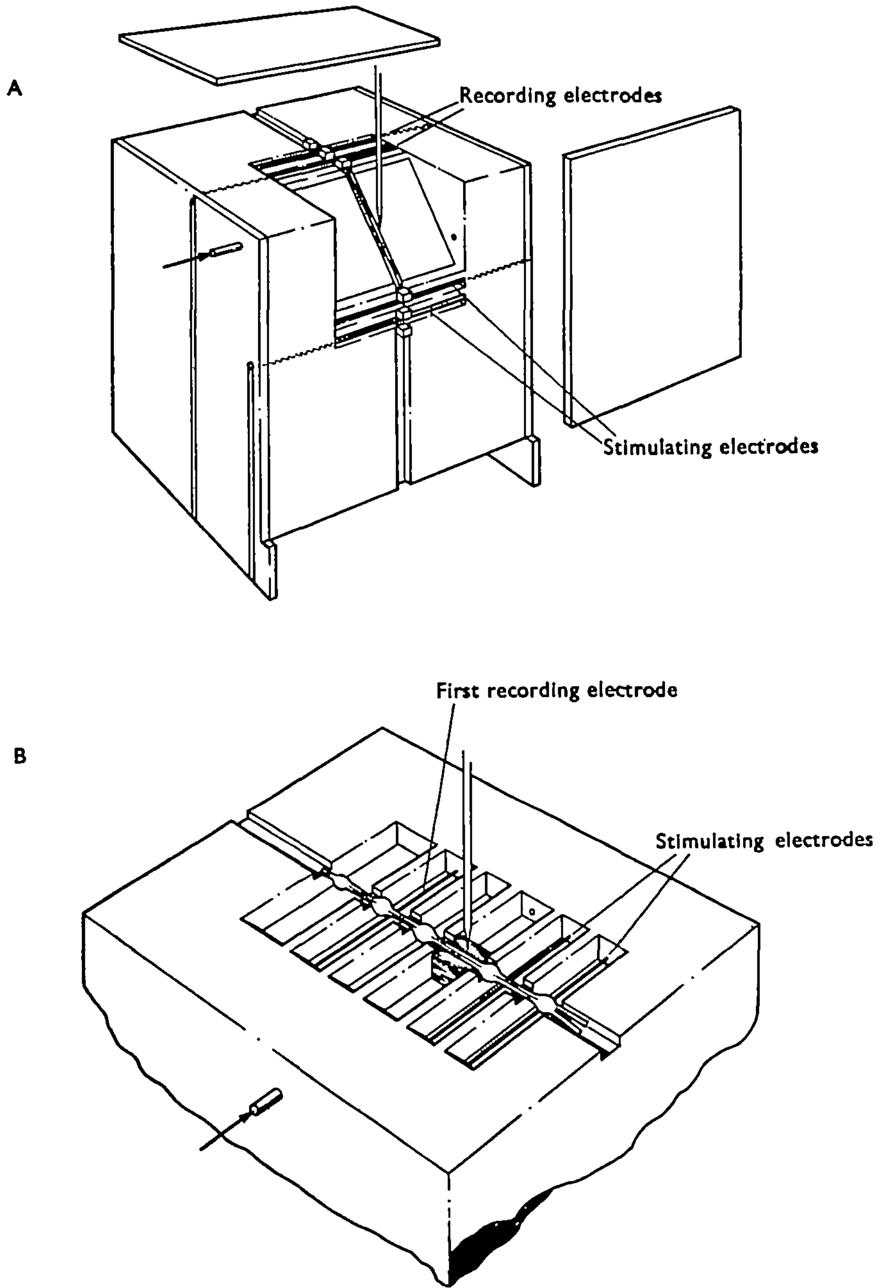


Text-fig. 1. Diagrams of the nerve chambers used in intracellular and extracellular recording of action potentials in penultimate connectives of the abdominal nerve cord. (A) In the first chamber, in which the connective was maintained under tension, the glass plates were pressed against the adjacent Perspex surfaces and maintained in position by a petroleum jelly seal. The small rubber cushions, with petroleum jelly, formed water-tight seals between adjacent compartments and also served to hold the nerve cord in position. The stimulating and recording wires were soldered to conducting strips on the two pieces of 'Veraboard'. These were connected to the outside circuit by two edge connectors, which also served to support the chamber. (B) In the second chamber the unstretched connective was supported on a small piece of foam rubber to facilitate penetration of the giant axons by the microelectrode. Petroleum jelly was used to form water-tight seals between adjacent compartments. As in the first nerve chamber the stimulating and recording electrodes were connected to the outside circuit by 'Veraboard' strips.

(1) 3% glutaraldehyde in 0·1 M cacodylate buffer (pH 7·4) with added 0·2 M sucrose, followed by washing in cacodylate buffer with sucrose and post-fixation in 1% osmium tetroxide in veronal acetate or cacodylate buffer;

(2) Karnovsky's (1965) formaldehyde-glutaraldehyde fixative in 0·1 M cacodylate buffer at pH 7·4 with added calcium chloride (0·25 mg/ml), followed by washing in cacodylate buffer with sucrose and post-fixation in 1% osmium tetroxide in cacodylate or collidine buffer. In both cases the tissues was dehydrated through an ascending series of ethanols and embedded in Araldite. Ultrathin sections were cut on a LKB Ultratome III, stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and examined in a Philips EM 200 or EM 300 electron microscope.

The connectives thus fixed and examined under the electron microscope were either in the intact or de-sheathed condition; in the latter instance their outer connective tissue sheath had been removed immediately prior to fixation.

The intracellular and extracellular recordings were carried out using isolated nerve cords mounted in the nerve chambers illustrated in Text-fig. 1. In the first of these (Text-fig. 1A) the nerve cord was maintained under appreciable tension, being stretched to approximately one and a half times the normal length. In the second nerve chamber (Text-fig. 1B) the connectives were supported on pieces of foam rubber without any application of tension, in order to facilitate penetration by glass microelectrodes. In both chambers stimuli were applied to the terminal pair of connectives by platinum wire electrodes, as an uninterrupted series of rectangular current pulses (0·5 sec^{-1}, 0·2 msec duration) at low output impedance via an RF isolating unit. Glass microelectrodes for intracellular recording were filled with 3·0 M-KCl and had resistances of between 10 and 30 M Ω and tip potentials of less than 5·0 mV. The microelectrodes were used in conjunction with a high-impedance F.E.T. input stage of unit gain, coupled to a Tektronix 532 or 561 oscilloscope. Extracellular recordings were made, via the platinum wire recording electrodes, using an Isleworth A101 pre-amplifier and a Tektronix 502A oscilloscope. Continuous recording of slow potential changes was made using a Smith Servoscribe potentiometric recorder.

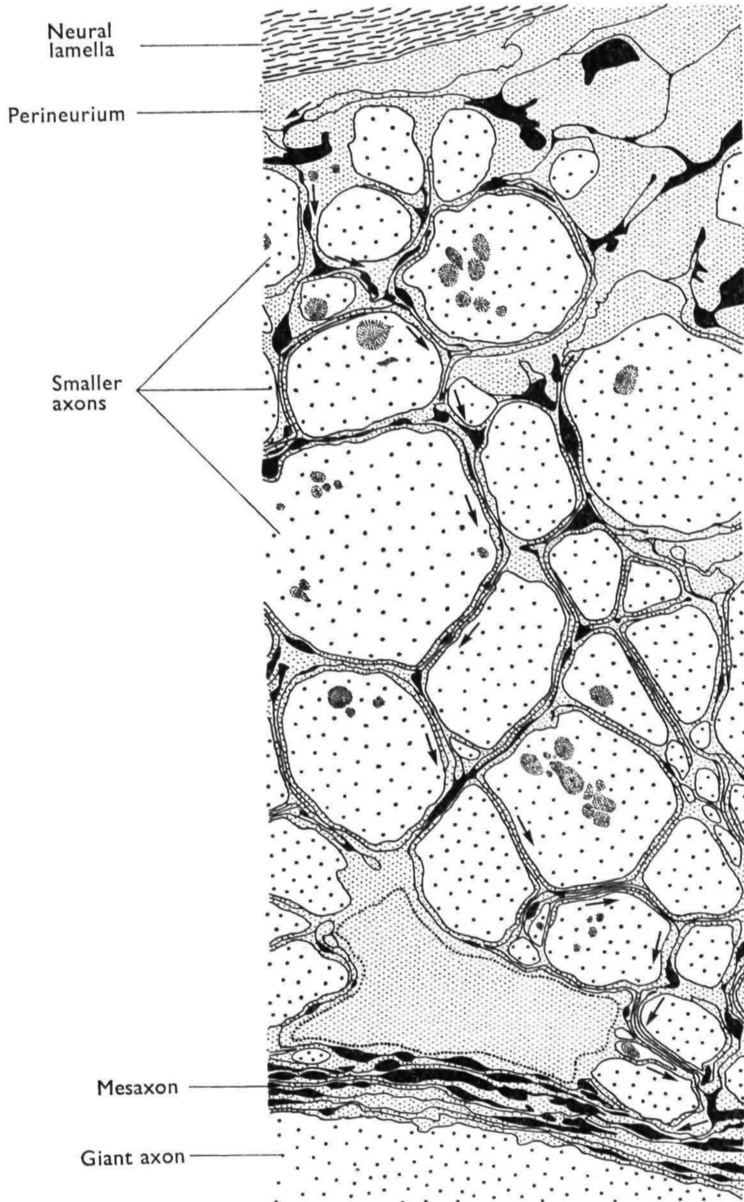
The nerve chambers were perfused by a gravity feed system, at a constant rate of 5–10 ml/min, from a series of elevated reservoirs. Rapid change of solutions with minimal mixing was achieved using a multiway non-return valve arranged close to the nerve chamber so as to reduce the dead-space. Experiments with dye solutions showed that the change of solution within the fluid compartment of the nerve chamber could be achieved within *c.* 2–5 sec.

De-sheathed connectives were prepared by placing an isolated nerve cord in a drop of saline under a binocular dissecting microscope. A small portion of the sheath was then torn away using two fine electrolytically sharpened tungsten needles. One connective of each pair was left intact, so as to give some mechanical support to the de-sheathed one when mounted in the nerve chamber.

The normal physiological solution used in this investigation was that devised by Yamasaki & Narahashi (1959) and had the following composition: 214·0 mM/l Na^+, 3·1 mM/l K^+, 1·8 mM/l Ca^{2+}, 216·9 mM/l Cl^-, 0·2 mM/l $H_2PO_4^-$ and 1·8 mM/l HPO_4^{2-}. Variations in the potassium concentration of the solution were accommodated by suitable alterations to the concentration of sodium ions.

Structural observations

The general organization of the cockroach central nervous system has been described by Smith & Treherne (1963) and an account of the structure of the perineurium has



Text-fig. 2. This diagram represents a cross-section of part of a cockroach connective including the outer connective tissue sheath or neural lamella, the perineurium, and the underlying axons with their ensheathing glial cells. It includes the edge of a giant axon which is surrounded by the spiral folds of the mesaxon. The extracellular spaces are indicated by the black lines and distended areas between the glia. The arrows alongside certain of these lines indicate one possibly pathway for diffusion of ions from the outer neural lamella to the beginning of the mesaxon channel; this particular pathway measures 20 μm in length.

been given by Maddrell & Treherne (1967). The description given here of the ultrastructure of the connective will therefore be confined to aspects concerned with quantitative interpretation of the electrophysiological data described in a succeeding section.

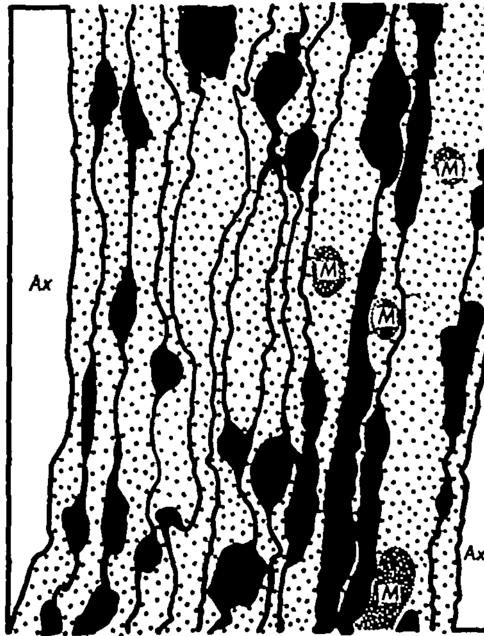
As indicated in Text-figs. 2. and Pl. 1, fig. 1 the periphery of the connective is delimited by the fibrous neural lamella. The underlying cellular layer, or perineurium, is channelled at the lateral borders of its component cells by tortuous intercellular clefts, *c.* 150 Å in width, which exhibit tight junctions and septate desmosomes at their inner ends.

Beneath the perineurium lie numerous axons of varying diameter, surrounded by glial cells. Certain of these, the giant axons, are extremely large and it is these with which the present investigation is concerned. The glia are separated from one another and from their axons by intercellular spaces; this extracellular system consists of a complex three-dimensional network of narrow intercellular channels with occasional larger spaces. It is extremely difficult to arrive at any quantitative description of this system. An approximate idea of the probable length of an intercellular channel linking the perineurium with the outer region of the glial cell of a giant axon is illustrated in Text-fig. 2. The length of this particular path is 20 μm. The glial cell associated with each giant axon is spirally arranged and is termed the mesaxon. A portion of a typical mesaxon is illustrated in Text-fig. 2 and Pl. 1, fig. 1, and in more detail in Text-fig. 3. The extra-axonal space is connected with the general extracellular system through the spiral mesaxon channel, which is *c.* 200 Å wide, but which has frequent dilatations, ranging from about 0·2 to 3 μm or more in length, in which the width of the cleft may be as much as 1500 Å. Measurements of high resolution electron micrographs yielded an average value of 235 Å for the mean width of the mesaxon channel associated with giant axons. The mesaxon spirals 6–7 times round these giant axons (see Pl. 1, fig. 1 Text-figs. 2, 3) and measurements from electron micrographs show that the length of an average mesaxon channel, from the extra-axonal space to the end of the spiral channel, is 770 μm. Tight junctions were frequently found between adjacent glial membranes in the mesaxon (Pl. 2, fig. 3). Such junctions represent localized regions of cell contact and do not occlude the intercellular pathway linking the extra-axonal fluid with the outer end of the mesaxon channel. These junctions have been shown to represent low-resistance pathways which may allow the penetration of relatively large (40,000 mol. wt.) molecules of peroxidase between adjacent glial elements (Lane & Treherne, 1969).

In longitudinal section (Pl. 3, fig. 4) it can be seen that each of the 6 or 7 glial cell processes ensheathing the giant axons continues without interruption for a considerable distance along the length of the axon. Although it was not possible to follow the giant axon along the entire length of the connective, because of grid-bar intervention, in those parts of the nerve cord examined no intercellular junctions could be observed in the glial cell process immediately adjacent to the giant axon. This suggests that the processes forming the spiral mesaxon cleft continue effectively without interruption by intercellular junctions. The assumption made in the theoretical section, that the mesaxon system approximately resembles an endless 'swiss roll', is thus justified morphologically.

Electron micrographs of de-sheathed preparations revealed that the removal of the

neural lamella also involved damage to the associated perineurium (Pl. 1, fig. 2). The ultrastructural appearance of the underlying tissues, however, did not appear to be appreciably affected by the de-sheathing procedure. In particular, the extent of the various extracellular spaces and the appearance of the axons and associated glial elements (Pl. 1, fig. 2) was essentially similar to that observed in intact connectives (Pl. 1, fig. 1), except for a slight distension of the glial cytoplasm in some of the peripheral cells.



Text-fig. 3. This diagram illustrates the extracellular channels and spaces which lie between the folds of the glial cell or mesaxon that ensheath each giant axon in a cockroach connective. The spaces on either side represent cross-sections of the axons (*Ax*) and there are six to seven ensheathing glial or mesaxon folds (stippled) around each of these. The black lines and distended areas represent the intercellular regions which range in width from 100 to 1500 A. Mitochondria (*M*) are shown in the glial cytoplasm.

THEORETICAL CONSIDERATIONS

The object of this section is to consider the theoretical behaviour of potassium ions in a simple model system, of structure analogous with that of the cockroach connective. The diffusion of ions in this system will be assumed to occur by the same mechanism as that in free solution, since the dimensions of the system are in general large compared with the hydrated ionic radius of the potassium ion, which may be taken as *c.* 2 Å (Stern & Amis, 1959). The effect of local restrictions, such as those at the inner ends of the intercellular clefts in the perineurium, has also been neglected for the sake of simplicity. In so far as the theoretical behaviour of the model system is quantitatively similar to the experimentally observed behaviour of the cockroach connective, it can thus be said that the diffusion of potassium ions in the intercellular spaces of the connective is restricted only by the geometry of the extracellular system and not by any specific mechanism.

In the intact connective, as is apparent from the previous section, the pathway for potassium ions diffusing inwards from the bathing medium to the surface of a giant axon may be divided into three main portions. These are, first, the complicated network of clefts between the small glial cells and peripheral axons; secondly, the long, spiral mesaxon channel around the giant axon; and thirdly, the narrow space between the innermost glial cell membrane and the axolemma itself, which functions to some extent as a reservoir in that it must be filled with ions in order to raise the mean concentration around the axon. The space immediately surrounding the axon will be referred to for convenience as the extra-axonal space. To render the problem tractable, the geometry of the model must necessarily be much simpler than that of the real system. The nature of the simplifications made will be discussed at each stage in the calculations. Assessment of the contribution to the diffusion time by each section of the complete pathway will be made by considering first the mesaxon channel alone, and then subsequently the effects of adding to the model system the parts representing the other sections of the pathway. A list of the symbols used in the calculations will be found at the end of the section (see p. 120).

The requirement in each case is to find a solution of the diffusion equation

$$\nabla^2 C - \frac{1}{D}\frac{\partial C}{\partial t} = 0, \qquad (1)$$

with boundary conditions appropriate to the geometry of the model. The solution is then used, together with the physical dimensions of the real system, to calculate the theoretical time-course of the change in the extra-axonal potassium concentration, following a step-change in the concentration in the bathing medium. For convenience it is assumed that the concentration everywhere in the system is initially close to zero; in the experimental situation, the initial concentration is 3·1 mM, which is small compared with the final concentration of 100 mM. The investigations are concerned only with the half-time for equilibration, which can be shown to be very little affected by this approximation. The step-change in the external concentration is assumed to take place at time $t = 0$.

The method of solution most conveniently used is to employ the Laplace Transform

$$\bar{C}(p) = \int_0^\infty C(x, y, z, t)\, e^{-pt} dt, \qquad (2)$$

so that the diffusion equation becomes

$$D\nabla^2 \bar{C} - p\bar{C} = C(x, y, z, 0), \qquad (3)$$

the appropriate solution of which is easily found. The remainder of the calculation then consists of algebraic manipulation of the solution, and the inversion of the transform. The procedure is lengthy; only the initial boundary conditions used and the final form of the solution in each case will be given here. The solution is generally in the form of an infinite series, chosen to give the most rapid convergence for large values of the time. For times close to the half-time, it is found that only the first two terms need be used.

(a) The mesaxon channel

The greater part of the diffusion pathway from the bathing solution to the axon surface is accounted for by the spiral cleft running through the mesaxon. The cleft is of variable width (Text-fig. 3), but both its radius of curvature and its length parallel to the axis of the system are large in comparison with its width.* It is thus effectively a straight, one-dimensional diffusion pathway, open at one end (chosen as the point $x = 0$), and feeding into the extra-axonal space at the other ($x = l$). As a first approximation the reservoir effect of the extra-axonal space will be neglected, so that the inner end of the tube will be considered to be closed. The effect of the variable cross-section of the cleft is difficult to assess; it might be considered that the frequent dilatations which are observed (Text-fig. 3) could act as reservoirs for ions diffusing along the cleft, the rate of diffusion being limited both by the narrowness of the constricted portions and by the necessity of filling the reservoirs. On the other hand, from longitudinal sections such as that of Pl. 3, fig. 4, it can be seen that very few individual dilatations extend for more than a short distance along the axis of the system; rather, the constrictions and dilations are to be regarded as localized in all three dimensions. Any dilatation thus has in parallel with it many areas of constricted cleft, and vice versa. Under these conditions the appropriate one-dimensional representation of the cleft is a tube of uniform cross-sectional area, equivalent to the mean width of the cleft. The mean width, determined from Text-fig. 3, is 235 Å. In that the dilatations may to some extent act as reservoirs, the half-time for diffusion, obtained from such a model, will be the minimum possible for a mesaxon channel of the given length.

With the above simplifications the boundary conditions for the problem are

$$C(0, t) = C_0 \text{ for all } t > 0, \tag{4}$$

$$\frac{\partial C}{\partial x}(l,t) = 0 \text{ for all } t \quad \text{(i.e. no flux at closed end)}, \tag{5}$$

$$C(x, 0) = 0 \text{ for all } x > 0. \tag{6}$$

The solution is given in two forms by Carslaw & Jaeger (1959), pp. 309–11

$$\frac{C_a}{C_0} = 1 + \sum_{n=1}^{\infty} \frac{4(-1)^n \exp[-D(2n-1)^2 \pi^2 t/4l^2]}{(2n-1)\pi}, \tag{7}$$

$$\frac{C_a}{C_0} = 2 \sum_{n=0}^{\infty} (-1)^n \operatorname{erfc}\left[\frac{(2n+1)\,l}{2\sqrt{(Dt)}}\right], \tag{8}$$

where C_a is the concentration at the inner end of the tube, adjacent to the axon.

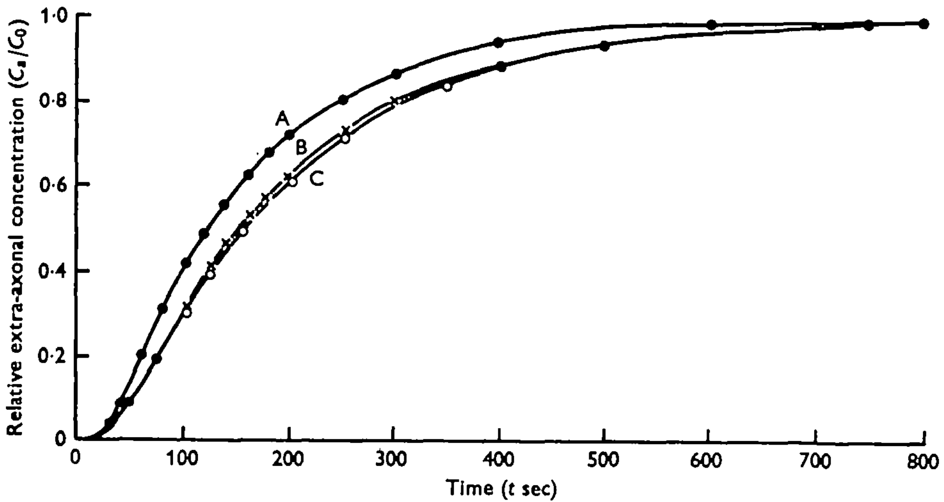
The form (7) is the more useful in the present case, since it has the more rapid convergence for large values of the time, although both forms converge quite rapidly around the half-time. The first term of (7) gives an approximate value for the half-time

$$t_{\frac{1}{2}} \doteqdot \frac{4l^2}{\pi^2 D} \ln \frac{8}{\pi}. \tag{9}$$

* The possibility exists that junctions, lying around the circumference of the axon, between adjacent mesaxon cells might occur frequently enough for the clefts thus formed to exert a 'short-circuiting' effect on the system. Examination of longitudinal sections (Pl. 3, fig. 4) suggests, however, that such junctions are not only infrequent, but also tortuous, so that it is unlikely that they have any significant effect.

The form (8) was that used by Nicholls & Kuffler (1964) for diffusion in the nerve cord of the leech. Both forms give a half-time which is independent of the width of the tube; the width becomes important, however, when the reservoir effect of the extra-axonal space is considered.

The measured length of an average mesaxon channel is 770 μm, corresponding to a half-time for diffusion of 124·8 sec (Curve (A) in Text-fig. 4).



Text-fig. 4. Predicted time-course of the change in extra-axonal potassium concentration (C_e): at time $t = 0$ the concentration in the bathing medium is raised instantaneously from zero to C_0. The three curves show the cumulative effects of three sections of the diffusion pathway as follows: (A) mesaxon channel alone, equation (7); (B) mesaxon channel with terminating reservoir formed by the extra-axonal space, equation (11); (C) as in (B), but with additional 20 μm intercellular pathway, equation (19).

(*b*) *The extra-axonal space*

The mesaxon channel feeds into a space, *c*. 200 Å wide, surrounding the giant axon. While the diffusion of ions around this space need not necessarily occur any more rapidly than it does in the mesaxon channel, the space will to some extent act as a reservoir for potassium ions since the electrical response of the axon will presumably be determined by the average concentration of ions in the space around it. Exchange of ions across the axolemma itself may also occur. In order to assess the maximum possible effect of the extra-axonal space, the assumption has been made that equilibration of potassium ions around the space is rapid, so that it functions as a perfectly stirred reservoir. The half-time for diffusion is thus increased; curve (B) in Text-fig. 4 is to be regarded as showing the outside limit of the effect.

The boundary conditions are:

$$-D \frac{\partial C}{\partial x} (l,t) = h \frac{\partial C}{\partial t}(l, t) \quad \text{for all} \quad t, \tag{10}$$

together with (4) and (6). h is a parameter equal to the ratio of the volume of the reser-

voir to the area of cross-section of the channel. The solution is (Carslaw & Jaeger, p. 129)

$$\frac{C_a}{C_0} = 1 - \sum_{n=1}^{\infty} \frac{2(1 + h^2\alpha_n^2) \sin\alpha_n l \exp(-D\alpha_n^2 t)}{\alpha_n[l(1 + h^2\alpha_n^2) + h]}, \tag{11}$$

where α_n is the nth. root of the equation

$$\alpha_n \tan \alpha_n l = 1/h. \tag{12}$$

As with the series (7), convergence is very rapid for large values of the time; the half-time is given approximately by

$$t_{\frac{1}{2}} \doteqdot \frac{1}{D\alpha_1^2} \ln \left\{ \frac{4(1 + h^2\alpha_1^2) \sin \alpha_1 l}{\alpha_1[l(1 + h^2\alpha_1^2) + h]} \right\}. \tag{13}$$

For an average giant axon the mesaxon channel is 770 μm long and 235 Å wide; the circumference of the axon is 110 μm, and the extra-axonal space is 200 Å wide, giving a value for the parameter h of $9{\cdot}36 \times 10^{-3}$ cm. The first root, α_1, of equation (12) has the value 18·19, and the half-time, determined from curve (B) of Text-fig. 4, is 154·9 sec.

(c) The outer glial pathway

The effect of the glial cells surrounding the mesaxon is to interpose a further network of narrow channels between the axon surface and the bathing medium. While the analysis of the behaviour of a complex network of channels is very difficult, an assessment of the possible effect can be made by considering only the shortest single route through the outer glial system. The pathway considered is indicated by arrows in Text-fig. 2, and has a length of *c.* 20 μm, and a width of the order of 200 Å. (As will be seen from Text-fig. 4C, the effect of this part of the system on the half-time is too small for any exact measurements to be worth while.) The problem of access of ions to the axon is thus that of diffusion in two consecutive channels of different diameters, terminated by the reservoir formed by the extra-axonal space. If the outer channel is considered to extend from $x = -b$ to $x = 0$, and the inner from $x = 0$ to $x = l$, the boundary conditions are

$$C_1 = C_2 = 0 \quad \text{for all} \quad x \quad \text{at} \quad t = 0, \tag{14}$$

$$C_1(-b, t) = C_0 \quad \text{for all} \quad t > 0, \tag{15}$$

$$C_1(0, t) = C_2(0, t) \quad \text{for all} \quad t, \tag{16}$$

$$a_1 \frac{\partial C_1}{\partial x}(0, t) = a_2 \frac{\partial C_2}{\partial x}(0, t) \quad \text{for all} \quad t, \tag{17}$$

$$-D\frac{\partial C_2}{\partial t}(l t) = h\frac{\partial C_2}{\partial t}(l t), \tag{18}$$

where C_1 and C_2 are the concentrations in the two channels, whose cross-sectional areas are a_1 and a_2, respectively. The inner channel opens at $x = l$ into a reservoir of volume equal to ha_2. The concentration at $x = l$ is then given by

$$\frac{C_a}{C_0} = 1 - \sum_{n=0}^{\infty} \frac{4 \exp(-D\beta_n^2 t)}{\beta_n\{\beta_n h[(1 + \mu)(b + l) \cos \beta_n(b + l) - (1 - \mu)(b - l) \cos \beta_n (b - l)] + (1 + \mu)(b + l + h) \sin \beta_n(b + l) + (1 - \mu)(b - l - h) \sin \beta_n (b - l)\}}, \tag{19}$$

where $\mu = a_2/a_1$, and β_n are the roots of

$$1 - \mu \tan \beta_n b \tan \beta_n l = \beta_n h(\tan \beta_n l + \mu \tan \beta_n b). \tag{20}$$

In the connective, $\mu = 235/200 = 1{\cdot}175$, $b = 20\ \mu\text{m}$, $l = 770\ \mu\text{m}$, $h = 9{\cdot}36 \times 10^{-3}\text{cm}$; thus $\beta_1 = 18{\cdot}0$. The half-time is obtained from Text-fig. 4, curve (C) and is 158·1 sec.

(*c*) *The de-sheathed system*

If it is assumed that removal of the sheath allows free penetration of ions into all glial cells, including the mesaxon, the problem of access to the axolemma becomes simply one of diffusion through a medium formed by the glial cytoplasm, followed by penetration through the intact innermost cell membrane of the mesaxon, into the small space surrounding the axon itself. As a first approximation, the system may be considered as one-dimensional; the glial cells are considered to extend from $x = 0$ to $x = l$, where they are bounded by a membrane of permeability P cm sec^{-1}, enclosing a space of depth h, around the axon. The boundary conditions are then

$$C(x,0) = 0 \quad \text{for all} \quad x > 0, \tag{21}$$

$$C(0, t) = C_0 \quad \text{for all} \quad t, \tag{22}$$

$$-D\frac{\partial C}{\partial t}(l, t) = P[C(l, t) - C_a] = h\frac{\partial C_a}{\partial t}, \tag{23), (24}$$

where C_a is, as before, the concentration of ions in the space around the axon.

C_a is then given by

$$\frac{C_a}{C_0} = 1 - \sum_{n=1}^{\infty} \frac{2h \exp(-D\alpha_n^2 t)}{\cos \alpha_n l\{(1 - h\alpha_n^2\delta)^2 + \alpha_n^2 h^2(l+\delta) + h\}}, \tag{25}$$

where α_n are the roots of

$$1 - h\alpha_n^2\delta = h\alpha_n \tan \alpha_n l, \tag{26}$$

and $\delta = D/P$.

The convergence of the series (25) is, as before, so rapid, except for very small values of the time, that only the first term need be considered. Also, for practical values of δ, l, and h, it is found that $\alpha_1 l$ is small, so that

$$1 - h\alpha_1^2\delta \doteqdot 0. \tag{27}$$

The behaviour of the system is then almost entirely dominated by the limiting effect of the membrane, and the behaviour of C_a with time becomes simply exponential

$$\frac{C_a}{C_0} \doteqdot 1 - \exp(Pt/h), \tag{28}$$

with a half-time given by

$$t_{\frac{1}{2}} \doteqdot \frac{h}{P} \ln 2 \tag{29}$$

For membrane permeabilities of the order of 10^{-6} cm sec^{-1} (cf. Hodgkin & Katz, 1949; Keynes, 1951; Hodgkin & Horowicz, 1959), the value of the half-time is about 1 sec (h = 200 Å). The experimental value of 24·0 sec thus suggests that, either the

membrane permeability is considerably lower than 10^{-6} cm sec^{-1}, or the rate of access of ions is partly limited by diffusion through the glial cytoplasm, or across the junctions between adjacent glial cells. If diffusion is to be partly rate-limiting in a system of this type, it is necessary to assume that diffusion takes place at a much slower rate than is the case in free solution. The requirement is that

$$\delta \sim D/P, \tag{30}$$

which, with $l = 15$ μm and $P = 10^{-6}$ cm sec^{-1}, implies an effective diffusivity of about 5×10^{-9} cm^2 sec^{-1}, as compared with $1 \cdot 8 \times 10^{-5}$ cm^2 sec^{-1} in free solution. If restricted access through the glial cells is considered to account for the experimental half-time for diffusion in the de-sheathed connective, it is thus clear that, although the tight junctions do provide a pathway for ions, the rate of passage of potassium ions through the pathway is severely limited. It is relevant to note that the experiments of Lane & Treherne (1969) with peroxidase, although they showed the possibility of access by relatively large molecules, provide no information on the actual rate at which the molecules penetrate the glial system. The point that emerges most clearly from both types of experiment is that the de-sheathing procedure does open an alternative channel, which by-passes at least part of the mesaxon and intercellular channels.

List of Symbols

$C(x, y, z, t)$ = instantaneous concentration of potassium ions at the point (x, y, z).

$C_1(x, y, z, t)$ = instantaneous concentration of potassium ions in the cleft between glial cells

$C_2(x, y, z, t)$ = instantaneous concentration of potassium ions in the mesaxon channel.

$C_a(t)$ = concentration of potassium ions at the axon surface.

C_0 = concentration of potassium ions in the bathing medium.

$\bar{C}(p)$ = the Laplace Transform of $C(x, y, z, t)$; p is a 'dummy variable'.

D = diffusion coefficient for potassium ions in aqueous solution,
= $1 \cdot 8 \times 10^{-5}$ cm^2 sec^{-1} (*Chemical Rubber Co. Handbook of Chemistry & Physics* 49th edition, 1968–9).

t = time.

l = length of mesaxon channel = 770 μm (also used in (*d*) for thickness of the outer glial layer, $\doteqdot$ 15 μm).

b = length of shortest pathway through the outer glial layer = 20 μm.

h = $\dfrac{\text{volume of extra-axonal reservoir}}{\text{cross section of channel feeding it}}$ = $9 \cdot 36 \times 10^{-3}$ cm (in §(*d*) the pathway through the mesaxon extends over the whole circumference, so that h becomes the thickness of the reservoir (= 200 Å).

a_1 = area of cross-section of channel between outer glial cells.

a_2 = area of cross-section of mesaxon channel.

$\mu = a_2/a_1 = 1 \cdot 175$.

α_n, β_n = nth roots of transcendental equations, quoted in the text.

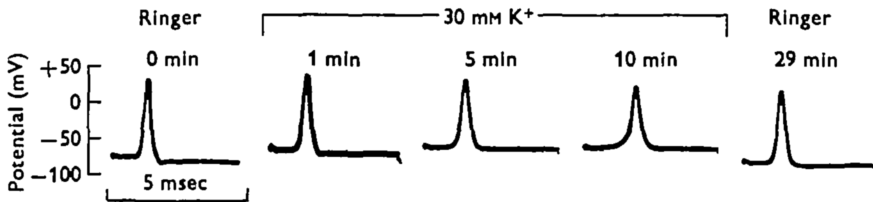
P = potassium permeability of the innermost glial cell membrane (cm sec^{-1}).

$\delta = D/P$.

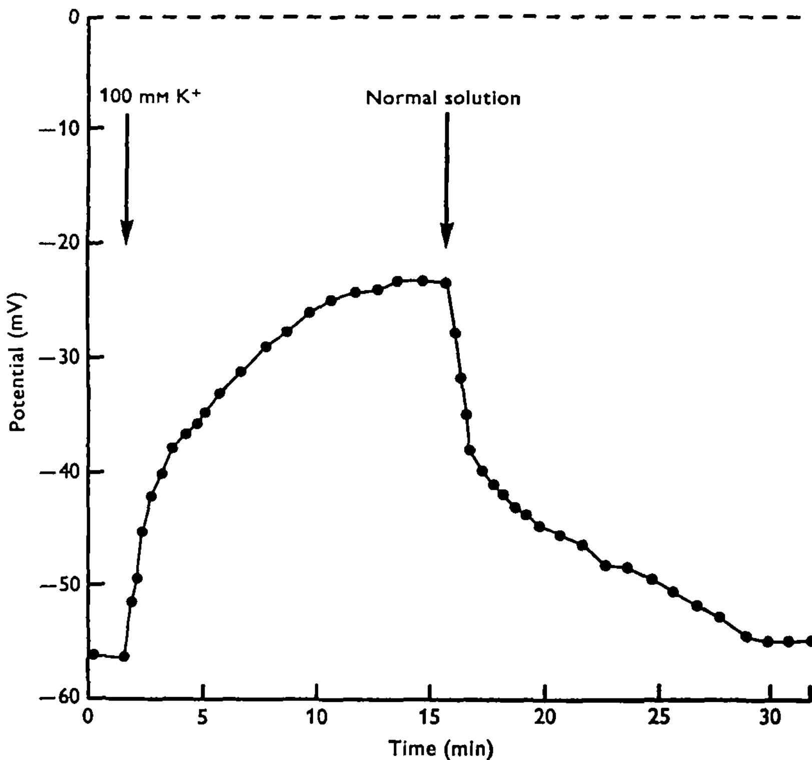
EXPERIMENTAL RESULTS

(*a*) *Intact, stretched preparations*

In intact preparations two essentially different electrical responses were obtained, following alteration in the potassium concentration of the bathing solution, according to the method employed in mounting the isolated nerve cords in the experimental chamber. We will consider first the results obtained with the connectives maintained



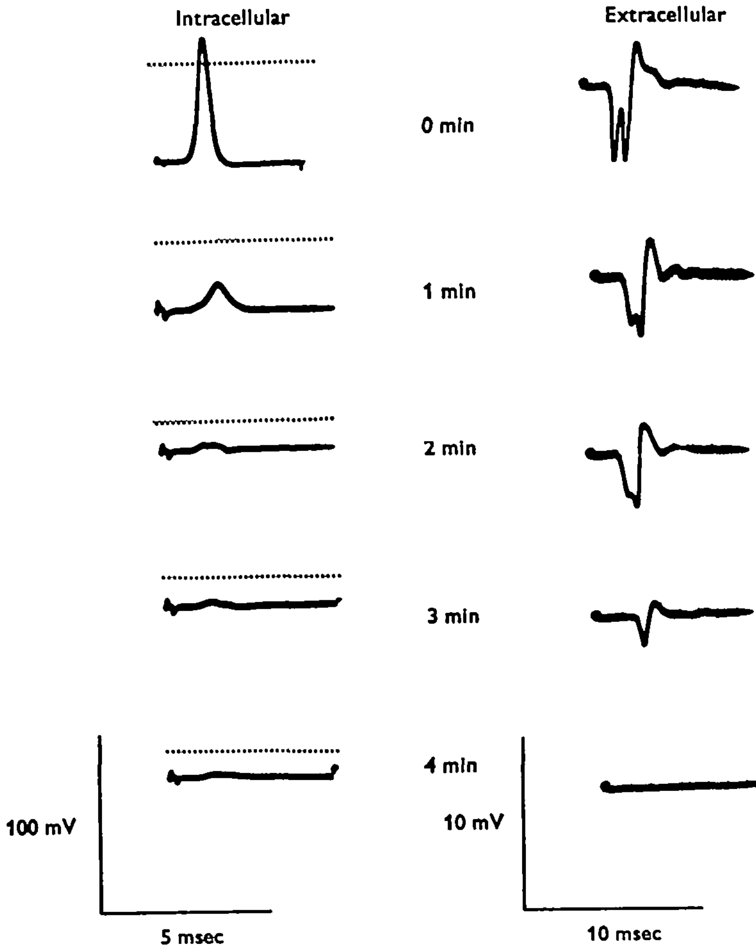
Text-fig. 5. Intracellular recording showing the effect of 30 mM/l K^+ on the resting potential of a giant axon in an intact stretched connective.



Text-fig. 6. The time-course of the depolarization, and subsequent repolarization, in a giant axon from an intact stretched preparation following exposure to a solution containing 100 mM/l K^+.

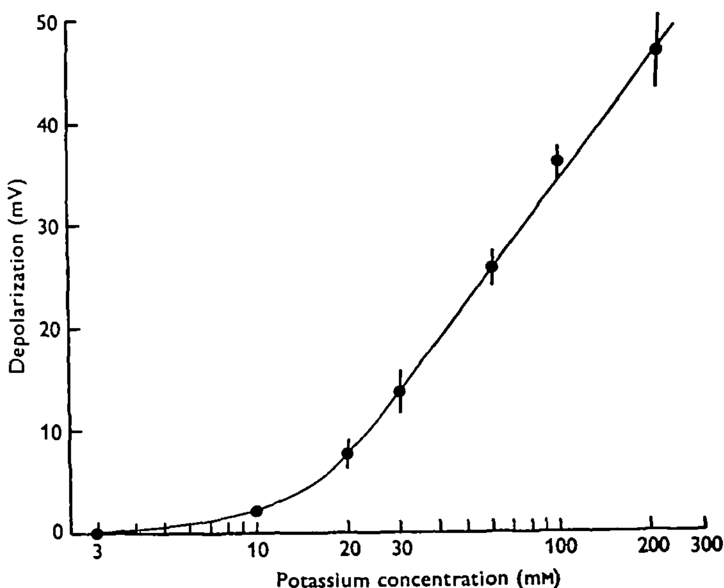
under tension in the nerve chamber illustrated in Text-fig. 1 A. This method, which involves stretching the connectives to approximately $1\frac{1}{2}$ times their unstretched length, is the conventional one usually adopted to facilitate penetration by microelectrodes.

With intact nerve cords maintained under tension the electrical response was of the conventional form illustrated in Text-fig. 5, that is to say there was a progressive reduction of the resting potential with no appreciable increase in the overshoot of the action potential. Text-fig. 6 illustrates the time-course of the depolarization and subsequent repolarization, measured during and after a period of exposure to a bathing solution containing 100 mm/l potassium.

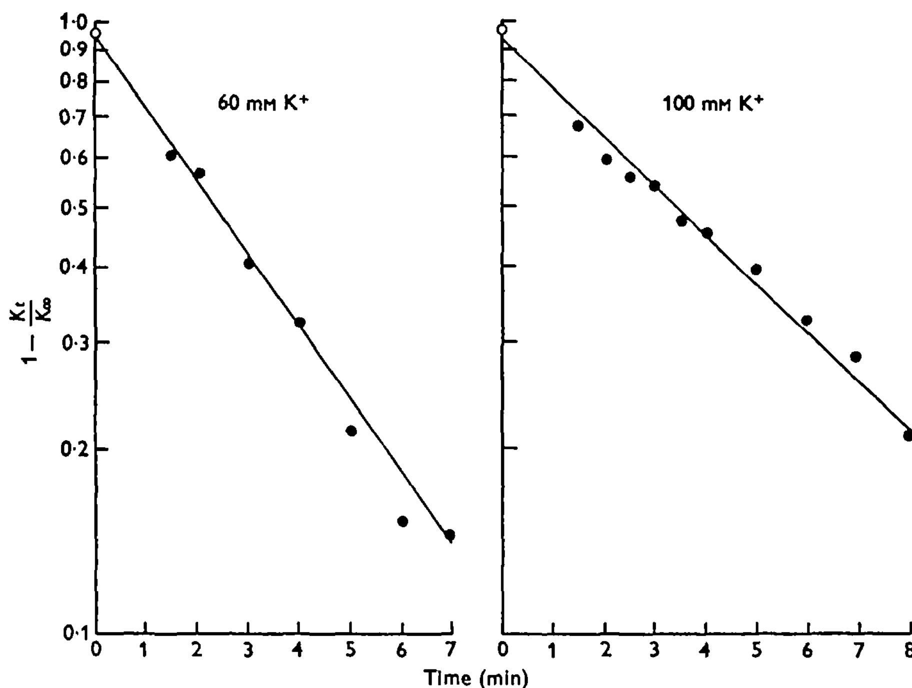


Text-fig. 7. Comparison of intracellular and extracellularly recorded action potentials following exposure of intact stretched connectives to an external potassium concentration of 100 mM/l.

To ensure that in these experiments the access of potassium ions to the axon surfaces was not increased as a result of damage caused to the overlying tissues by the microelectrode, the rate of depolarization was compared using intracellular and extracellular recording techniques in stretched preparations. It will be seen from Text-fig. 7 that the intracellularly recorded action potentials paralleled that obtained using extracellular electrodes. It seems reasonable to assume, therefore, that the penetration of the central nervous tissues by the microelectrode did not appreciably increase the access of potassium ions to the surface of the impaled axon.



Text-fig. 8. The relation between depolarization in giant axons and the potassium concentration of the bathing medium in intact connectives. The exponential portion of the lines has a slope of 42 mV for tenfold change of concentration. Each point represents the mean of five observations; the vertical lines represent the extent of twice the standard error of the mean.



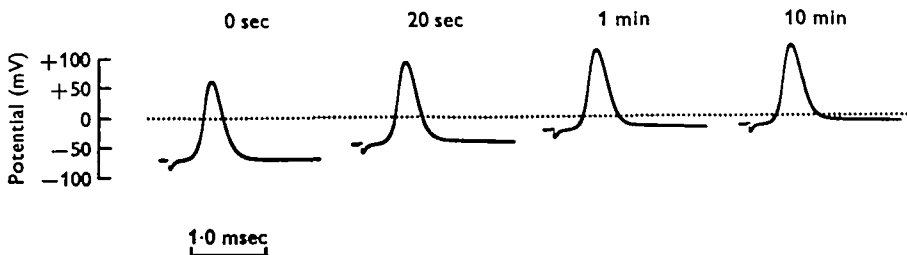
Text-fig. 9. The calculated change in the relative concentration of potassium ions at the surface of giant axons in stretched connectives following increase in the concentrations of potassium ions in the bathing medium to 60 and 100 mM/l. These values were obtained from depolarization measurements using the data illustrated in Text-fig. 8.

The relationship between the external potassium concentration and depolarization in axons from intact stretched preparations, is illustrated in Text-fig. 8. The exponential portion of the graph has a slope of 42 mV per ten-fold change of concentration, which is identical with that quoted by Yamasaki & Narahashi (1959) for the relation between potassium concentration and resting potential in giant axons of de-sheathed cockroach nerve cords. In stretched preparations no significant difference was observed in the present investigation between the level of depolarization of axons in intact and in de-sheathed connectives. With an external solution of 100 mM/l, for example, the depolarization was found to be 35.9 ± 1.1 mV in intact nerve cords which is not significantly different from the mean value of 34.8 ± 4.9 mV measured in de-sheathed ones. It seems reasonable to suppose, therefore, that the changes in potassium concentration in the external-axonal fluid are quantitatively related to the level of this cation in the external medium.

Using the data shown in Text-fig. 8 it is possible to calculate the rate of change in potassium concentration in the extra-axonal fluid from the depolarization curves obtained following an alteration of the external concentration of this cation (Text-fig. 6). Only the linear portion of the graph, relating depolarization to the external potassium level, was used in the estimation of the extra-axonal concentration. It will be seen from Text-fig. 9 that the apparent rate of change of concentration of potassium in the fluid bathing the axon surfaces in stretched preparations declined in an exponential manner as would be expected from a first-order diffusion process. The mean half-time for the experiments, carried out with external concentrations of 60 and 100 mM/l potassium, was 173.0 ± 22.6 sec.

(*b*) *Intact, unstretched preparations*

With unstretched preparations (mounted in the apparatus illustrated in Text-fig. 1 B) the electrical response following alterations in the potassium concentration of the bathing medium was fundamentally different from that previously described for the

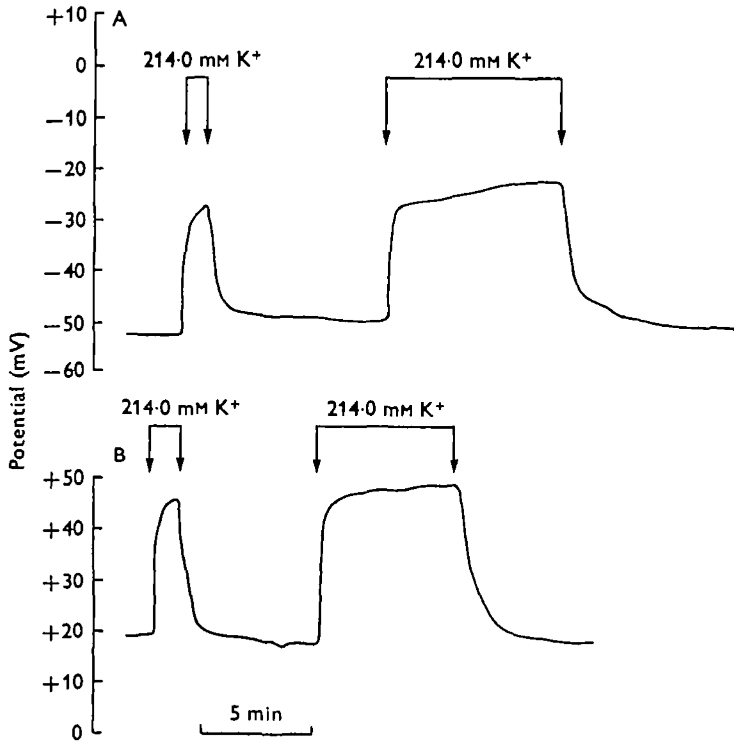


Text-fig. 10. Intracellularly recorded action potentials (from giant axons in an intact unstretched connective) following exposure to a bathing solution in which the sodium was replaced by potassium ions.

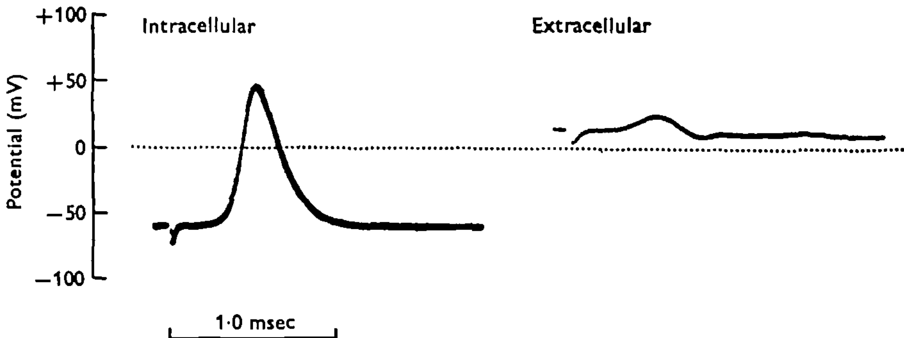
stretched preparations. Text-fig. 10 illustrates the effects of a sodium-free solution, containing 214.0 mM/l potassium, on the action potentials recorded with this method of mounting the nerve cord. It will be seen that the apparent depolarization was not associated with any equivalent reduction of the intracellularly recorded action potentials. Text-fig. 11 illustrates the alteration in the potential, measured with an

intracellularly located microelectrode, during a period of exposure of an unstretched connective to a high-potassium saline. This result shows the typical and extremely rapid apparent depolarization, which was followed by a plateau.

However, when the microelectrode tip was withdrawn into an apparently extracellular position (Text-fig. 12), the form of the electrical responses was essentially



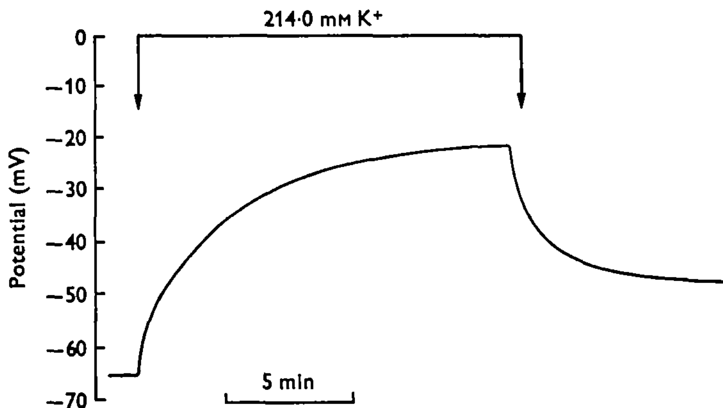
Text-fig. 11. Continuous recordings of potential changes obtained during exposure of intact unstretched connectives to a high-potassium/sodium-free solution. (A) Intracellularly recorded potential from a giant axon; (B) potential changes recorded with the tip of the microelectrode in an extracellular position (see Text-fig. 12).



Text-fig. 12. The electrical responses obtained with the microelectrode tip located within a giant axon following withdrawal into an apparently extracellular position. The latter recordings show the biphasic form of the action potential and the appreciable positive potential associated with an extracellularly located microelectrode (Pichon & Boistel, 1967).

similar to that obtained with intracellularly located electrodes (Text-fig. 11 B). It seems clear from this, and from the other results obtained with the unstretched connectives, that the apparent depolarization of the giant axons observed with high external potassium concentrations did not represent a reduction of the resting potential, but arose from the interpolation of an additional positive extracellular potential.

The electrical response resulting from the elevation of the external potassium concentration, using the nerve chamber in which connectives were supported on pieces of foam rubber (Text-fig. 1 B), could be converted to the form obtained in the first nerve chamber (Text-fig. 1 A) by the application of tension to the preparation (Text-fig. 13).



Text-fig. 13. Continuous recording of potential changes observed in an impaled giant axon, associated with an increase in the concentration of potassium ions in the bathing solution, in a stretched preparation mounted in the nerve chamber illustrated in Text-fig. 1 B.

(c) *De-sheathed preparations*

In de-sheathed preparations, both stretched and unstretched, a very rapid depolarization was observed following alteration in the external concentration of potassium ions (Text-fig. 14). A pronounced asymmetry was observed between the depolarization and the repolarization associated with a period of exposure to solutions containing elevated potassium concentrations. A similar effect has been described in the glial cells of the leech, *Hirudo medicinalis*, where the repolarization also proceeded at a slower rate than the initial depolarization (Nicholls & Kuffler, 1964).

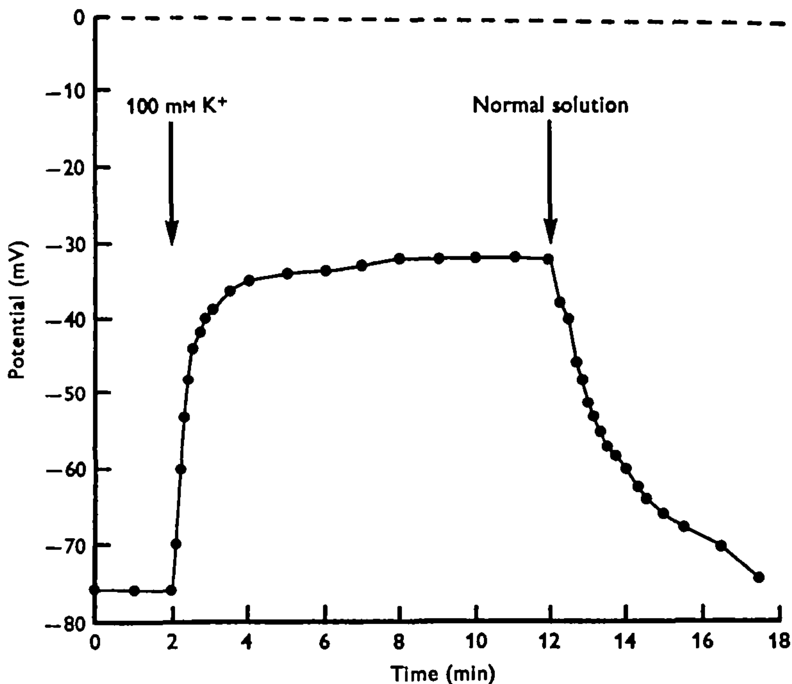
As with stretched intact preparations the rate of change of potassium concentration in the fluid bathing the axon surfaces varied in an exponential manner, following increase in concentration of external potassium ions (Text-fig. 15). The measured half-time for change in the extra-axonal potassium concentration was found to average $24 \cdot 0 \pm 5 \cdot 2$ sec in de-sheathed preparations.

DISCUSSION

This study was undertaken in an attempt to elucidate the nature of the processes involved in the exchanges of inorganic ions between the blood (or bathing medium) and the fluid immediately surrounding the giant axons within intact connectives. A particular aim of this investigation was the quantitative estimation of the role of

various structural elements in the movement of inorganic cations between the periphery of the nervous system and the surface of axons contained in the complex structural milieu of the central nervous tissues.

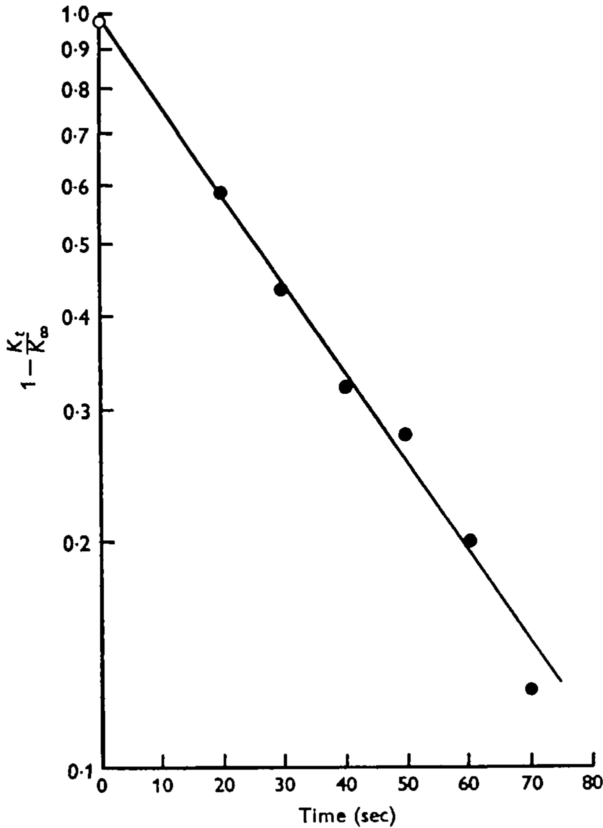
The essential structural features which are relevant to this analysis have been described in a preceding section and can be summarized as follows. The immediate fluid environment of the giant axons consists of a 200 Å layer delimited by the innermost mesaxon glial membrane. This extra-axonal fluid compartment is confluent with



Text-fig. 14. The changes in resting potential of a giant axon associated with the exposure of a de-sheathed connective to a solution of 100 mM/l K^+.

the spiral mesaxon channel which discharges at the surface of the mesaxon into the overlying extracellular system. The latter consists, in essence, of a complex three-dimensional network of channels formed by the closely applied glial and axonal membranes. The periphery of the connective is surrounded by the nerve sheath, which consists of a superficial connective tissue layer and an underlying cellular layer. The latter is characterized by the presence of tortuous lateral channels, which frequently exhibit tight junctions at the inner margin.

It is apparent from the experimental results that the preparations used in this investigation fall into three classes, each class showing a distinct type of electrical behaviour in relation to changes in the concentration of potassium ions in the bathing medium. The results will accordingly be considered under the three headings: 'intact, stretched', 'intact, unstretched' and 'desheathed'. Clearly, the 'intact, unstretched' preparation represents the closest approach to the physiological situation, while the other two types have been subjected to varying degrees of deliberate interference.



Text-fig. 15. Rate of change of relative potassium concentration in the fluid bathing the surface of a giant axon, following an increase in the external concentration of this cation to 100 mM/l, in a de-sheathed connective.

(a) *Intact, stretched preparations*

This type of preparation will be considered first since, as has already been pointed out, it represents the method of treatment conventionally employed in the investigation of the electrical properties of cockroach axons. It is clear from the results described above that the potential changes recorded in intact, stretched preparations, unlike those recorded in unstretched preparations, represent a true depolarization of the giant axons in response to elevation of the potassium concentration in the bathing medium. It is thus reasonable to interpret the results from stretched connectives in terms of the access of ions by diffusion to the neuronal surface.

There are two routes by which potassium ions could reach the extra-axonal fluid from the external medium: access to the axon surfaces could be achieved by diffusion along the extremely narrow intercellular channels, or, alternatively, the ions could diffuse within the glial system. The latter process would involve the penetration of at least two glial membranes, the one at the external perineural surface and the inner mesaxon membrane adjacent to the giant axon. The glial cytoplasm forms an appreciable proportion of the volume of the central nervous tissues, and would impose

a significant reservoir effect in the movement of ions between the external medium and the extra-axonal fluid. The minimum diffusion path through the glia could thus be represented by a system consisting of a relatively large aqueous reservoir bounded on two margins by membranes. Diffusion through such a system can be described by an equation of the following form:

$$\frac{C_a}{C_0} = 1 - \frac{\alpha+\beta}{2\alpha} \exp \frac{P(\alpha-\beta)t}{2hl} + \frac{\alpha-\beta}{2\alpha} \exp \frac{P-(\alpha+\beta)t}{2hl},$$

where $\alpha = (4h^2 + l^2)^{\frac{1}{2}}$ and $\beta = 2h + l$, and the other symbols have the same meanings as used in the theoretical section, with l equal to the separation between the two membranes, whose potassium permeabilities have been assumed, for simplicity, to be the same. Taking the thickness, l, of the glial layer as 15 μm, and the membrane permeability, P, as 10^{-6} cm sec^{-1}, the half-time for diffusion as given by the above equation is 2120 sec. The fact that the experimental half-times, calculated from depolarization curves, in stretched preparations have at most one-tenth of this value, suggests that the access of ions is principally via the extracellular channels rather than through the glial cytoplasm. This conclusion, which is similar to that reached by Nicholls & Kuffler (1964) for leech ganglia, is further supported by the consideration that access of ions through the glial cells, whose membrane potential is maintained by a Donnan system, initially involves disturbance of the chloride equilibrium of the cells, followed by potassium chloride movement (Boyle & Conway, 1941) and is therefore controlled by the chloride permeability, as well as by the potassium permeability of the cell membranes. Glial cells in such species as have been studied (Kuffler & Potter, 1964; Kuffler, Nicholls & Orkand, 1966) appear to have low chloride permeabilities.

The theoretical analysis of the diffusion processes in the intercellular pathways indicated that the major component, in the system linking the extra-axonal fluid with the periphery of the connective, was represented by the extended mesaxon channel. It was shown that the half-time for the diffusion of potassium ions along a mesaxon channel of 770 μm in length lay between 125 and 155 sec, depending upon the precise form of model used, as compared with the experimentally measured half-time of 173·0 sec for the movement of potassium ions from the bathing medium to the axonal membranes in stretched preparations.

It is extremely difficult to give a precise quantitative analysis of diffusion through the complex system of intercellular channels lying between the mesaxon and the surface of the connective. However, it has been shown that the addition to the mesaxon channel of a single intercellular diffusion pathway (equivalent to that connecting the opening of the mesaxon channel to the periphery) only resulted in a slight increase in the calculated half-time, bringing it to a value of 158 sec.

It seems clear, from the above comparison of the theoretical and experimentally determined values, that there is little appreciable restriction upon the diffusion of potassium ions in the intercellular channels linking the extra-axonal fluid with the periphery of the stretched connective. Access to the surfaces of the giant axons from the external medium appears, in fact, to be largely determined by the length of the diffusion pathways, especially that formed by the mesaxon channel. These conclusions

are in essential agreement with the results obtained for the exchange of radioactive ions and molecules between the central nervous tissues and the bathing medium (Treherne, 1961, 1962, 1965).

(b) *Intact, unstretched preparations*

In unstretched preparations the experimental results indicate that a very different situation exists as compared with that in preparations which have been stretched during the mounting procedure. It is clear that the very rapid and dramatic apparent depolarization obtained, following elevation of the potassium concentration of the bathing medium, did not result from a significant reduction of the resting potential of the giant axons. The most reasonable explanation of this phenomenon would seem to be that a diffusion potential is established between some portion of the extracellular system and the bathing medium. Recent experiments carried out in this laboratory (Y. Pichon & J. E. Treherne, in preparation) suggest that this extra-neuronal potential could result from the more rapid penetration of potassium into the extracellular system, relative to the outward diffusion of sodium ions down the concentration gradient created by substitution of a sodium-free, high-potassium bathing solution.

The presence of such an extra-neuronal potential, together with the relatively slow depolarization of the giant axons observed in response to elevation of the external potassium concentration, indicates that a considerable degree of restriction must occur in the penetration of small water-soluble cations to the axon surfaces in unstretched preparations. The relatively rapid exchanges of radioactive isotopes observed in cockroach nerve cords (Treherne, 1961, 1962, 1965), together with the observation that even quite large peroxidase molecules (mol. wt. 40,000) can pass through the superficial connective tissue sheath (Lane & Treherne, 1969) suggests that the apparent restriction to ion movements does not result from any degree of impermeability of this structure. It has also been shown that peroxidase molecules can penetrate into the intercellular clefts of the perineurium, and are only restricted at the inner end of these channels (Lane & Treherne, 1970) in the region containing tight junctions and septate desmosomes demonstrated in an earlier investigation (Maddrell & Treherne, 1967). It seems most likely, therefore, that the diffusion potential created by the reversal of the sodium/potassium ratio of the bathing medium in unstretched preparations may be associated with this region of the intercellular channels which traverse the perineurium. It should, however, be emphasized that at this time the possibility cannot be excluded that the effect might result from the presence of the acid mucopolysaccharide in the narrow intercellular channels or from the tight junctions which have been shown to occlude some regions of the mesaxon channels associated with the giant axons.

The question now arises as to the route by which potassium ions gain access to the extra-axonal fluid in intact, unstretched preparations, where there is an appreciable restriction to extracellular diffusion from the bathing medium. It has been calculated, in a preceding paragraph, that diffusion of potassium ions by an intracellular route through the glial system would occur with a half-time which would be at least an order of magnitude greater than that for the unrestricted movement through the intercellular channels which seems to occur with stretched preparations. It follows, therefore, that it is impossible on the present evidence to distinguish between the situation

in which access to the extra-axonal fluid occurs by a relatively slow intercellular diffusion, or by intracellular movement through the glial system.

It is apparent, as has already been mentioned above, that the restricted movement of potassium from the bathing medium to the extra-axonal fluid in intact, unstretched preparations does not correspond to the relatively rapid exchange demonstrated using radioactive isotopes (Treherne, 1961, 1962). The radioactive ion fluxes were measured using isolated preparations, in which the restriction upon ion movements appears from the electrical evidence to have been abolished. It is significant in this respect that recent experiments using the sucrose-gap technique (Y. Pichon & J. E. Treherne, in preparation) have shown that drying of the connectives with filter paper and subsequent exposure to air results in a significant reduction in the measured extraneuronal potential obtained with a raised potassium concentration in the bathing medium, together with a faster rate of depolarization of the giant axons. As previous investigations using radioisotopes involved the removal of superficial solution by drying on filter paper before measuring ion efflux, it follows that these earlier experiments, although giving quantitative information on the state of extracellular inorganic ions, provided an incomplete picture of the exchange processes occurring in the nervous system of the living animal. Further investigations of radioisotope exchange using modified experimental procedures are being currently pursued in this laboratory.

(*c*) *De-sheathed preparations*

As in a previous investigation (Twarog & Roeder, 1956), it has been shown that the de-sheathing procedure greatly increased the accessibility of the axons to potassium ions from the bathing medium, both in stretched and unstretched preparations. The increased rate of depolarization produced by removal of the peripheral nerve sheath was found to correspond to a half-time of 24·0 sec for the movement of potassium ions between the external medium and the extra-axonal fluid, as compared with 173·0 sec in the intact, but stretched preparations. As the mesaxons do not appear to be directly damaged by the de-sheathing procedure, it can be concluded that in de-sheathed preparations the mesaxon channels do not form the major pathway for the diffusion of ions to the extra-axonal fluid, for the measured half-time of 24·0 sec is only a fraction of that calculated for the diffusion of potassium ions along the length of the mesaxon channel. It follows from this that the de-sheathing procedure opens up an additional diffusion channel which permits a more rapid access of ions to the axon surfaces. Some recent investigations from this laboratory have supplied evidence as to the probable nature of this diffusion pathway. It has been shown, for example, that the surgical removal of the connective tissue portion of the nerve sheath necessarily involves changes in the underlying cellular layer, the perineurium. Thus a local removal of the nerve sheath renders the underlying glial system accessible to the large (mol. wt. 40,000) molecules of horseradish peroxidase (Lane & Treherne, 1969). The observations also suggest that the peroxidase might pass from glial cell to glial cell via tight junctions between adjacent glial membranes. These observations do, in fact, supply structural evidence for the low-resistance pathways linking glial cells demonstrated electrically in the nerve cord of the leech (Kuffler & Potter, 1964). The demonstration of these low-resistance glial connexions also makes it possible to explain the massive increase in the measured inulin space in de-sheathed nerves

(Treherne, 1962) for electron micrographs of such preparations did not reveal any significant increase in the extracellular spaces as compared with intact nerve cords. It is clear, therefore, that in de-sheathed preparations the diffusion pathway to the axon surfaces must be drastically reduced. As local tight junctions occur between adjacent mesaxon membranes it would also seem that the extended mesaxon channel would be short-circuited. The potassium ions diffusing by such a route from the external medium might thus only have to traverse a single cell membrane, that is the glial membrane immediately adjacent to the axon surface. The question thus arises as to whether the permeability of a single glial membrane would allow a rate of access sufficient to explain the rate of accumulation of potassium ions demonstrated in de-sheathed preparations in this study. Such a system has been examined quantitatively in a preceding section. In this analysis an unrestricted access of ions was assumed to the inner glial fold from the surface of the connective. It was found that with such a system access of ions to the neuronal surface would be sufficiently rapid to account for the observed rate of depolarization, even if the membrane permeability were as low as 10^{-9} cm sec^{-1}. This is much lower than the permeability given, for example, by Hodgkin & Katz (1949) for squid axons, which is $1 \cdot 8 \times 10^{-6}$ cm $sec.^{-1}$. Thus diffusion from a relatively high intracellular level in the glial cytoplasm could account for the observed rate of movement of potassium ions to the axon surfaces in de-sheathed preparations.

In comparing the behaviour of the de-sheathed connective with that of the intact connective it should also be noted that the function of the glial cell is profoundly altered by the de-sheathing procedure. In the intact system the intracellular potassium concentration is high, and the glial resting potential is likely to be close to the potassium equilibrium potential (cf. the situation in the leech (Kuffler & Potter, 1964) and in *Necturus* (Kuffler *et al.* 1966), where the glial cell membranes behave as almost perfect potassium electrodes). The potassium content of the glial cells will thus be maintained by a Gibbs–Donnan equilibrium, with little or no net flux of potassium ions across the cell membranes. If the glial resting potential is lower than the potassium equilibrium potential, however, there will be a net passive efflux of potassium ions from the glial cells, which will affect the extra-axonal concentration. For example, if the glial cell membrane has a potassium permeability of 10^{-6} cm sec^{-1}, a resting potential of -75 mV, and a potassium equilibrium potential of -80 mV, the equations of Hodgkin & Katz (1949) can be used to show that there will be a net potassium efflux of about $2 \cdot 5 \times 10^{-12}$ mol cm^{-2} sec^{-1}. If these ions are confined in a space only 200 Å thick surrounding the axon, the concentration in the space will rise at the rate of 1 mM/l. every second. As has been shown in the theoretical section, this rate of change is comparable with that which can be brought about by passive diffusion of ions through the intercellular clefts from a medium of high concentration, even in stretched preparations. Escape by passive diffusion thus cannot occur fast enough to balance such a rate of efflux from the glia, without a considerable rise in the extra-axonal concentration. It must therefore be postulated that, in the steady state, ions leaking from the axon and glia either do so at an extremely slow rate, or are taken up by the linked sodium-potassium exchange pumps in the axonal and glial cell membranes sufficiently rapidly to prevent any significant accumulation. If the latter is the case inhibition of the sodium pump, for example by exposure to ouabain, will initiate a rapid depolarization of the axons, owing to accumulation of potassium ions in the

extra-axonal space. While there is no evidence on this point for the cockroach, neurones of the snail, *Helix aspersa*, whose extracellular spaces are similarly restricted (Treherne & Moreton, 1970), maintain their resting potentials for periods of an hour or more after treatment with ouabain (R. B. Moreton, in preparation). Leakage from the glia thus appears to have little effect on the extra-axonal potassium concentration.

In the theoretical section the behaviour of the intact system was analysed on the assumption that the movements of ions occurred in narrow intercellular spaces with impermeable walls. It has also been shown that this model system correctly predicts the movements observed in stretched preparations. If the efflux of potassium ions from the glia is restricted by the low permeability of the glial cell membranes, then this assumption is correct. If, on the other hand, exchange of ions across the glial membranes occurs relatively freely, the behaviour of the system could be considerably modified. The effect of exposure of the connective to a potassium-rich solution is initially to raise the potassium concentration adjacent to the outermost glial layer; as diffusion proceeds, more deeply-lying glia and axons, and eventually the giant axons themselves, become exposed to the high potassium concentration. The effect of this is to depolarize the cells, tending to reduce the potassium efflux from them, since their resting potentials will more closely approach the potassium equilibrium potential (Hodgkin & Katz, 1959; Moreton, 1968). Entry of potassium chloride may also occur, owing to the raised KCl product of the outside solution (Boyle & Conway, 1941), although this effect is likely to be small, since the chloride permeability of glial cells has been shown to be very low (Kuffler & Potter, 1964; Kuffler *et al.* 1966). At the same time, there is no reason why uptake of potassium ions by the sodium pump should not continue, since it is unaffected by changes in the resting potential (Hodgkin & Keynes, 1955). Exposure of the connective to a raised concentration of potassium ions could thus have the effect of substantially reducing the efflux of potassium from the glial cells, or even of converting it into a net potassium influx. This would have the effect of creating a reservoir for potassium ions, whose effective size, depending on the rate of exchange across the cell membranes, could be such as to increase the diffusion time by a factor of 2 or more. The fact that the experimentally determined half-time for diffusion in stretched preparations is in good agreement with the theoretical value, together with the evidence that potassium efflux from the glia in another nervous system of similarly restricted extracellular space is normally small, suggests that diffusion within the intercellular clefts of the cockroach connective is not significantly retarded by exchange with the intracellular potassium.

In the de-sheathed system, the interior of the glial cells is made freely accessible to relatively large molecules. The greater part of the intracellular anions and cations will thus be lost to the bathing medium, and the Gibbs-Donnan system formed by the glial cell membranes will be destroyed. The effect of raising the potassium concentration of the bathing medium is then simply to cause influx of potassium ions into the outer layer of cells, followed by diffusion through the glial cytoplasm, up to the innermost glial cell membrane, which has been shown to be the first effective barrier. Penetration into the extra-axonal space then occurs at a rate depending on the concentration difference across this membrane, and the permeability of the membrane to potassium ions. As has been shown in the theoretical section, penetration to the axon surface occurs more rapidly in a system of this type than in the

intact stretched system, even if diffusion through the glial cells is considerably restricted.

SUMMARY

1. Using the electrical response of giant axons in the isolated abdominal nerve cord of the cockroach, *Periplaneta americana*, as an indication of the ionic composition of the fluid bathing their surfaces, it has been shown that the movement of potassium ions from the bathing medium to the extra-axonal fluid, following an increase in the external concentration of this cation, involves an appreciable degree of restriction.
2. This effect is associated with an extracellular diffusion potential, which appears to result from the more rapid penetration of potassium relative to the outward diffusion of sodium ions from the extracellular system.
3. It is suggested that the restriction of intercellular diffusion may occur in the region containing tight junctions and separate desmosomes at the inner end of the intercellular clefts which traverse the perineurium.
4. If the connectives are stretched during mounting, a more rapid depolarization of the giant axons is observed. Comparison of the calculated and the experimentally observed half-times for diffusion of potassium ions to the axon surface indicates that in these preparations the rate of movement of inorganic ions from the external medium is largely determined by the extended intercellular diffusion pathway represented by the mesaxon cleft.
5. In de-sheathed preparations penetration of potassium ions is still more rapid, an effect which is postulated to result from damage to the perineurium, and the consequent production of a shorter, intracellular diffusion channel through the glial system.

We are grateful to Mrs C. A. Beaumont for her help in carrying out some of the electrophysiological experiments, to Miss Y. Carter for assistance with electron microscopy and photography, and to Mr J. W. Rodford for preparing the illustrations used in this paper. Our thanks are also due to Dr E. R. Lapwood for his helpful advice in mathematical matters, and Dr P. Asher for stimulating discussions during the course of this work.

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EXPLANATION OF PLATES

PLATE 1

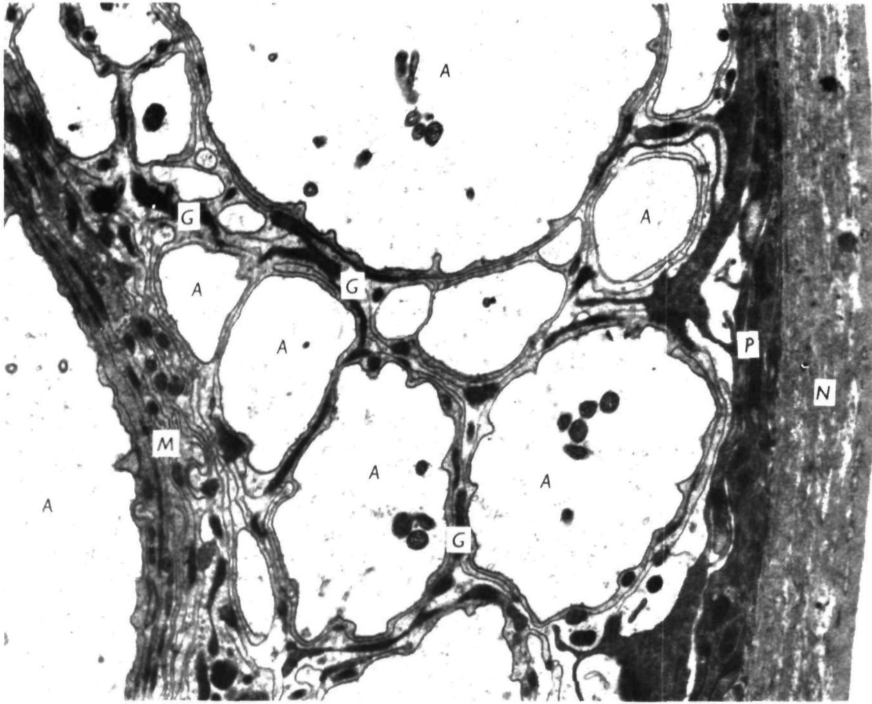
Figs. 1, 2. Both these electron micrographs are cross-sections of connectives from the abdominal nerve cord of the cockroach, *Periplaneta americana*. In both cases the neural lamella and perineurium lie to the right, ensheathing the axons and glia which comprise the interior of each connective. In fig. 1 the connective is intact, while that in fig. 2 has been de-sheathed. In the latter the point at which the connective tissue sheath was removed can be seen at the lower edge of the micrograph as can the underlying perineurium which is in a somewhat damaged condition; the glia and axons, however, appear fairly similar to those in the intact connectives. These tissues have been fixed in 3 % glutaraldehyde in cacodylate buffer with added sucrose, followed by postosmication and embedding in Araldite. The sections were stained in uranyl acetate and lead citrate. N, Neural lamella; *P*, Perineurium; *A*, Axons; *G*, Glia; *M*, Mesaxon round the giant axon. Fig. 1, × 10,000; fig. 2, × 400.

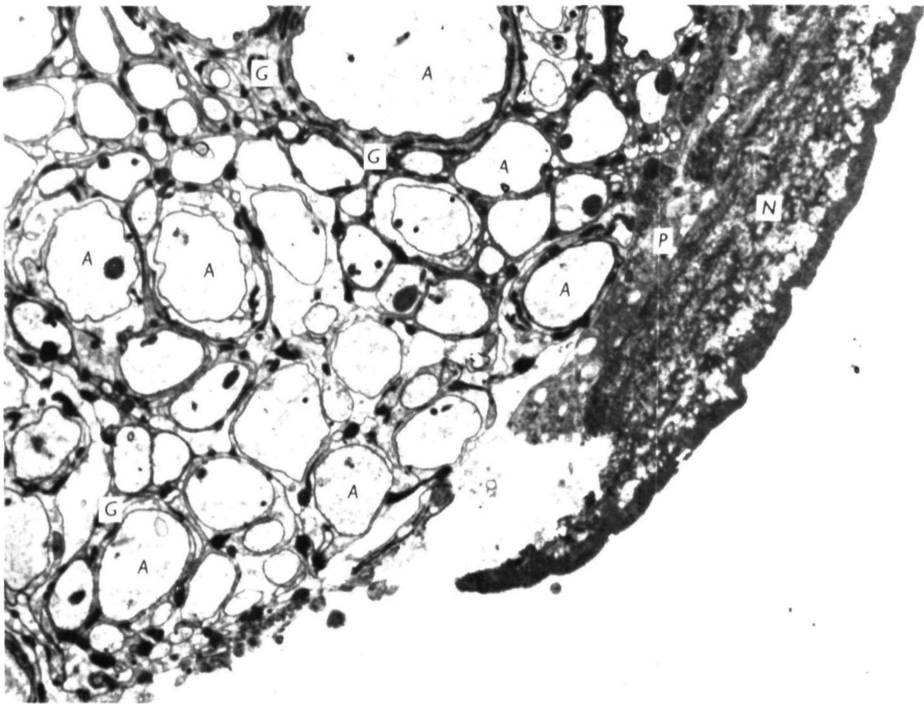
PLATE 2

Fig. 3. This electron micrograph shows part of a cockroach connective fixed in a formaldehyde-glutaraldehyde solution. The tight junctions are indicated by arrows. These junctions lie between glial cells which in turn ensheath the axons; the latter are identifiable by their content of neurotubules. This section was stained with uranyl acetate and lead citrate. *G*, Glial cells; *A*, Axons; *E*, Extracellular space, × 153,000.

PLATE 3

Fig. 4. A typical longitudinal section through a cockroach connective in the region of a giant axon (*A*), showing the layered glial cells that constitute the mesaxon (*M*). Note that there are few if any interdigitations of other glial processes interrupting the continuity of each glial cell layer. Hence it may be assumed that the 'swiss roll' arrangement of glial layers around the giant axons, as seen in cross-sections like fig. 1, is continuous over a considerable distance. The extracellular spaces, which in some cases form dilatations (*E*) between glial cell membranes, do not extend for more than short distances along the axon. × 25,000.





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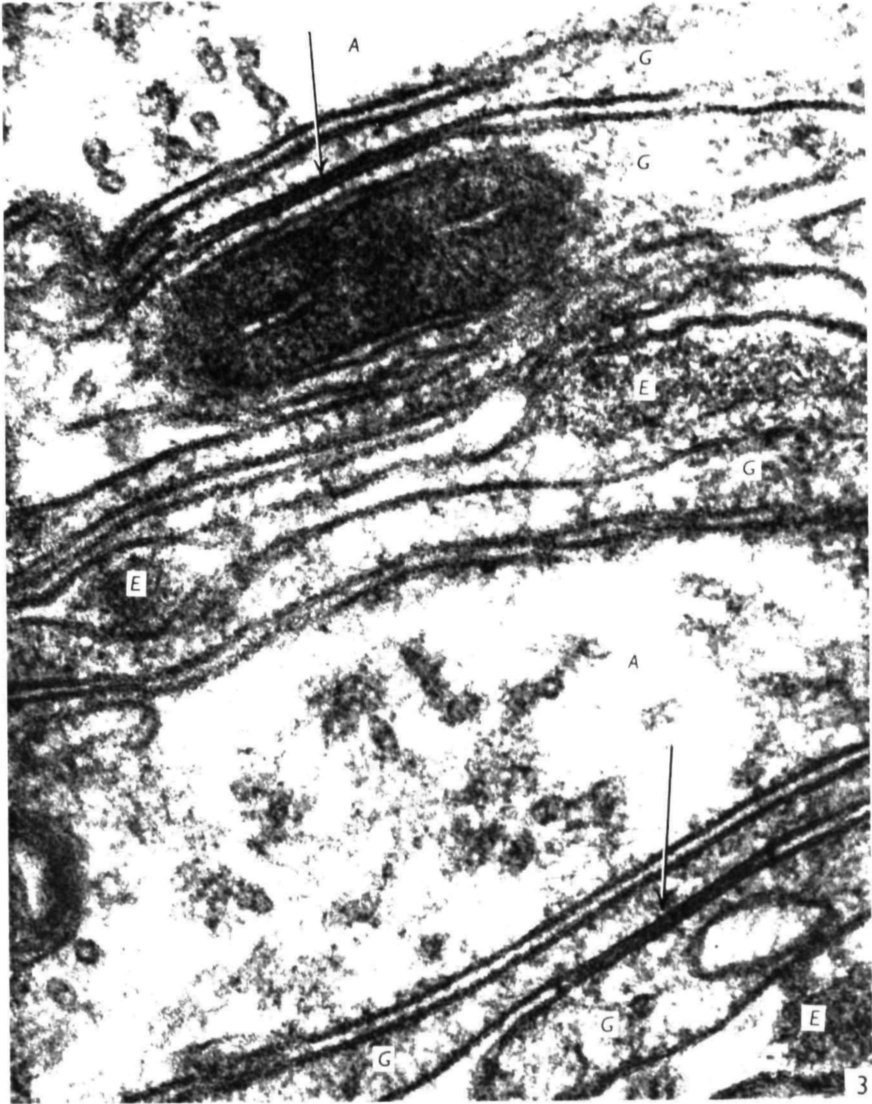
(*Facing p.* 136)



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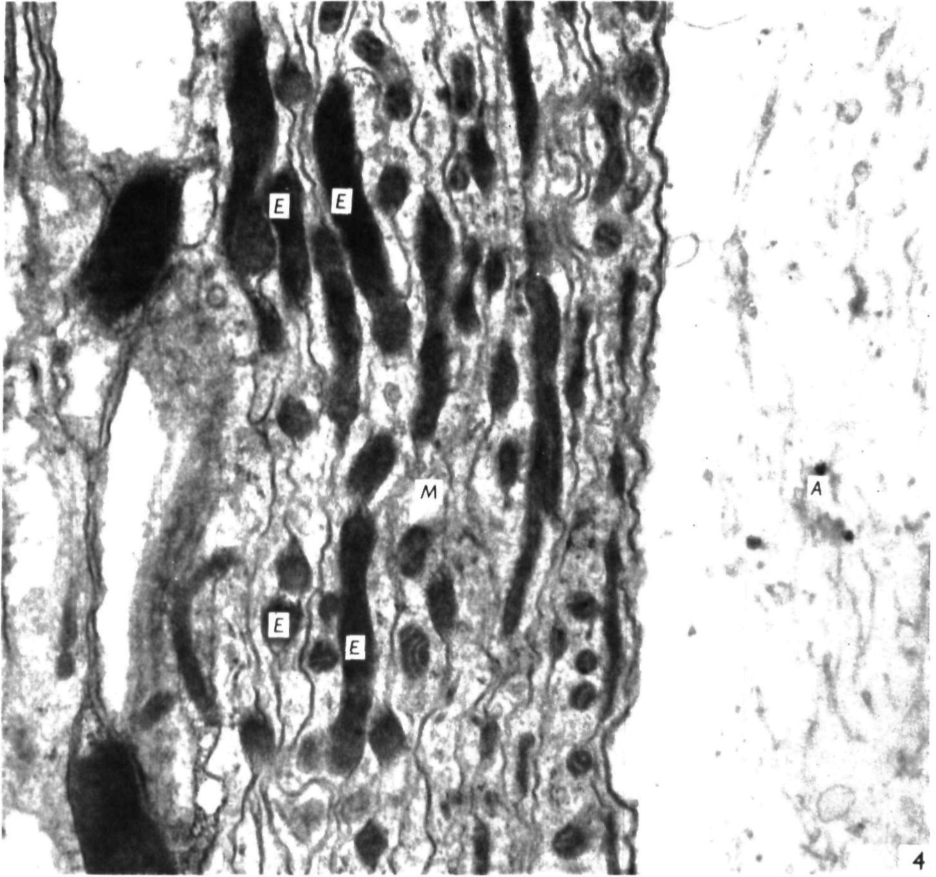

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