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The local chemical environment of nodes of Ranvier: a study of cation binding

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INTRODUCTION

The periodic annular constrictions described by Ranvier (1871) along the course of peripheral myelinated nerve fibres excited the interest of many later investigators of peripheral nerve structure and function. Subsequent light microscope investigations of these 'nodes of Ranvier' culminated in the careful study of Hess & Young (1952), who paid particular attention to the dimensions and chemical characteristics of the perinodal space and the structures which give rise to the 'cross of Ranvier'. The last is a cruciform staining of the nodal region produced when peripheral myelinated nerve fibres are immersed in aqueous silver nitrate and subsequently exposed to sunlight (Ranvier, 1875, 1878): similar appearances have been obtained using other metal salts (Macallum & Menten, 1906). Hess & Young demonstrated that, by pressure on the coverslip covering their teased nerve fibre preparations, the Ranvier cross could be separated into two components. They considered that the longitudinal component represented the nodal portion of the axis cylinder, and the transverse one represented a cementing disc, which surrounded the exposed portion of the axon at the node and was composed of 'scleroprotein' material continuous with the neurilemma. They concluded that the axon at the node was probably not covered by Schwann cell cytoplasm. Further, they commented that staining of the disc by silver nitrate and methylene blue raised the suspicion that 'coloration is due to active ionic interchanges perhaps with a high concentration of chloride and potassium ions', and that the composition of the disc 'must have some effect on the passage of ions and other substances from the axon surface to the perinodal space'. These observations appear to have passed largely unremarked by most workers concerned with electrophysiological studies of the role of the node in the process of saltatory conduction (Hodgkin, 1964; Tasaki, 1968).

Electron microscopical studies have demonstrated that the morphological relationships of axon and Schwann cell at the nodes of peripheral nerve fibres are considerably more complex than had been envisaged from light microscope observations, and that this morphology becomes more elaborate and more ordered with increase in fibre diameter (Gasser, 1952; Elfvin, 1961; Williams & Landon, 1963; Landon & Williams, 1963; Berthold, 1968). Consistent features of the structure of the nodes of the larger (> 6 μm) myelinated nerve fibres include crenation of the paranodal myelin sheath with fluting of the subjacent axon, and the accumulation of columns of Schwann cell cytoplasm, rich in mitochondria, within the external grooves of the myelin sheath. On

the nodal side of the paranodal bulb these cytoplasmic columns fuse to form a terminal collar of cytoplasm from which there projects a regularly ordered array of microvillous processes directed radially inwards to end in close relationship to the nodal axon membrane. The microvillous fingers lie embedded in an amorphous, moderately electron-dense, extracellular material, the gap substance, which fills the nodal gap deep to the confluent Schwann cell basement membranes. These structural features are illustrated diagrammatically in Fig. 1. An understanding of how this morphology is related to function, and specifically to ionic movements during the passage of the action potential, clearly requires a detailed knowledge of the physico-chemical properties of the elements which contribute to the environment of the axolemma at the node.

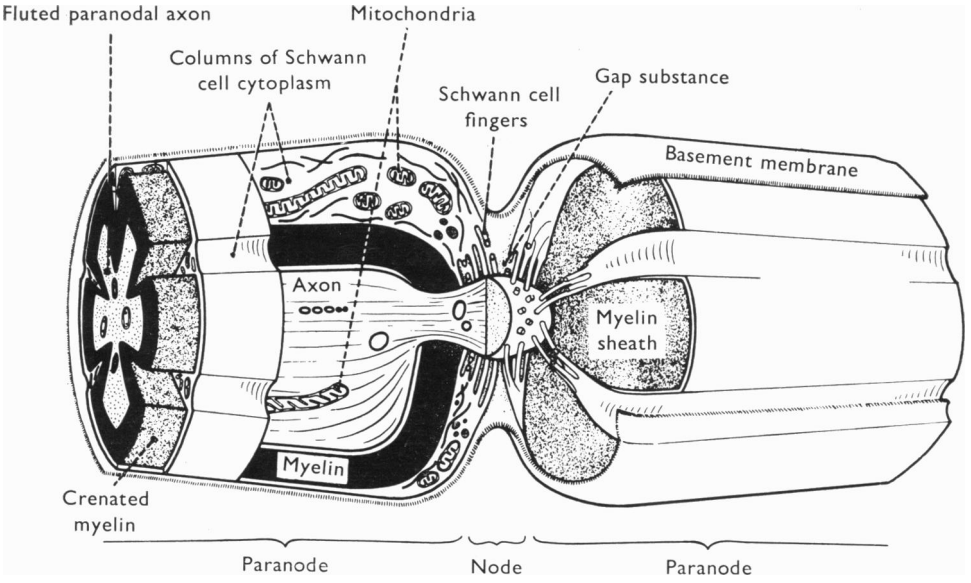


Fig. 1. A diagrammatic representation of the structure of a node of Ranvier of a large mammalian peripheral myelinated nerve fibre. (P. L. Williams and D. N. Landon, in *Gray's Anatomy*, 34th ed. London: Longman, Green, 1967, p. 62.)

In recent years several workers have demonstrated the affinity of the nodes of Ranvier, and of the cementing disc in particular, for a wide variety of metallic cations (Herbst, 1965; Gerebtzoff & Mladenov, 1967). This affinity has provided grounds for criticism of the specificity of certain histochemical methods which have been claimed to demonstrate the presence of acetyl cholinesterase at the nodes of Ranvier (Zenker, 1964; Langley & Landon, 1969). Some indication as to the nature of the mechanism responsible for this ion binding capacity was provided by the observations of Abood & Abul-Haj (1956) that a local concentration of non-sulphated mucopolysaccharides exists at and around the nodes of peripheral nerve fibres. It appeared to us of interest to determine, first, the fine structural location of these mucopolysaccharides and the extent to which they represented the chemical counterpart of the gap substance, and secondly, the relative affinities of different ionic species for the binding sites at the node. Our initial attempts to answer these questions included a study of the fine

structural localization of the Hale colloidal iron stain at the nodes of rat peripheral nerve fibres (Langley & Landon, 1967), and a light microscope study of the cation exchange properties of the nodal region in fixed and unfixed rat sciatic nerves (Langley, 1969).

The present report describes an extension of this work to the fine structural localization of the binding sites for certain of these cations and some preliminary observations on the binding of the polycation Alcian blue to nodes at varying salt concentrations. A preliminary report of these observations has already appeared (Landon & Langley, 1969).

MATERIALS AND METHODS

Sciatic nerves were dissected from adult rats anaesthetized with ether. The nerves were fixed in Palade's osmium tetroxide fixative (Palade, 1952) for periods of 1–2 h. The perineural sheath was removed after fixation. Hale staining was performed as described previously (Langley & Landon, 1967). Binding of Fe^{3+} Ba^{2+} and K^+ was accomplished by immersion of fixed nerves in solutions of the chlorides at concentrations of 0·01–0·1 M (Langley, 1969). Nerves were subsequently washed in distilled water (3 × 5 m). Tissue bound Fe^{3+} was then revealed with 0·01 M acidified potassium ferrocyanide, Ba^{2+} was precipitated either with 0·2 % sodium rhodizonate or as the sulphate with 0·1 N sulphuric acid, and K^+ was localized with ice-cold 1 M sodium cobaltinitrite followed by rinsing in cold water and conversion to black cobalt sulphide with dilute aqueous ammonium sulphide.

Copper binding was studied by the immersion of osmium tetroxide fixed nerves for 1 h in solutions containing copper sulphate (0·004–0·1 M) and equimolar sodium citrate at a pH ranging from 4 to 7 (Langley & Landon, 1969). After washing in distilled water, the bound cupric ion was demonstrated by its conversion to the pigment Hatchett's brown by treatment with potassium ferrocyanide.

Staining with Alcian blue was achieved by immersion of fixed nerve for at least 3 h in 0·01 or 0·1 % aqueous solution buffered at pH 5·7 with 0·05 M sodium acetate. Critical electrolyte concentrations of different cellular elements were assessed on 7 μm cryostat sections of fixed nerve with concentrations of sodium or magnesium chlorides in the staining solution in the range 0·1–1·3 M.

Lanthanum staining was studied on nerve fixed in Palade's fixative to which 1 % La $(NO_3)_3$ had been added. Ruthenium red was similarly included at a concentration of 0·1 % in 3 % glutaraldehyde phosphate buffered at pH 7·2 as primary fixative. After 30 min post-fixation was effected with 1 % buffered osmium tetroxide containing 0·1 % ruthenium red.

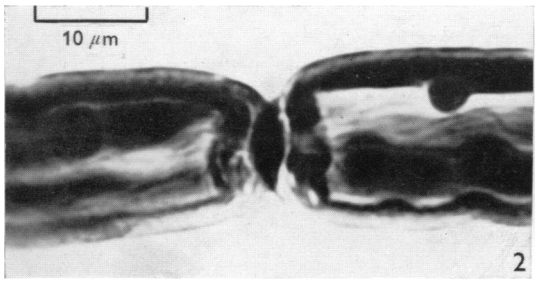
Fixed stained nerves were teased apart with fine needles and mounted in 30 % glycerine for light microscopy. For electron microscopic examination nerves were dehydrated through graded alcohols and embedded in TAAB epoxyresin. Thin sections cut with glass knives were mounted on Formvar support films and examined without further staining at 100 kV in an RCA EMU 3G electron microscope.

The effects of enzymic digestion with trypsin, testicular hyaluronidase, neuraminidase and phospholipase C, and the effects of chemical blockade of acidic groups with methanolic HCl were studied as described earlier (Langley & Landon, 1967; Langley, 1969).

RESULTS

Ferric ion and the Hale stain

The light microscopical appearances of the nodes of Ranvier of fixed peripheral myelinated nerve fibres stained by the Hale colloidal iron method (Pearse, 1963) were in all essentials similar to those of fibres immersed in 0·01 M ferric chloride and subsequently treated with potassium ferrocyanide solution. In both cases the region of the nodal gap contained a dense deposit of Prussian blue (Figs. 2, 3), which in sectioned material could be seen to form a ring around the nodal axon. Electron microscopy, however, revealed significant differences in the site of deposition of this pigment. Whereas the Hale staining moiety appeared to be restricted to a narrow zone spanning the nodal axon membrane (Fig. 4, and Langley & Landon,1967), the bound ferric ion was found to be wholly extra-axonal, the stain being distributed as a



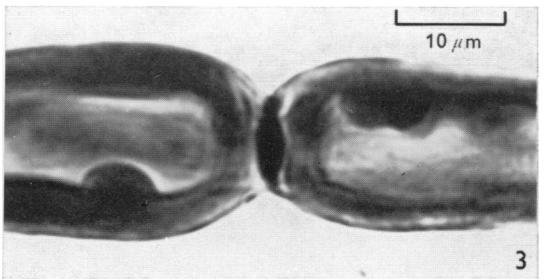


Fig. 2. A teased osmicated nerve fibre stained by the Hale colloidal iron technique. A dense bar of Prussian blue pigment is visible at the node.

Fig. 3. A similar osmicated nerve fibre following treatment with 0·01 M ferric chloride. The bound ferric ions have been precipitated as Prussian blue yielding an almost identical dense bar across the node.

finely granular precipitate throughout the whole nodal gap substance, and as coarser crystals in the adjacent endoneurium (Fig. 5). The quantity and extent of the latter component increased with increase in staining time.

Barium ion

Barium ion bound at nodes of Ranvier was demonstrated for light microscopy by its conversion to either the insoluble pink salt barium rhodizonate or to its insoluble sulphate. In the former case the nodes were surrounded by a ring of the small needle-like crystals of barium rhodizonate, with some larger crystals scattered throughout the endoneurium; in the latter case phase-contrast microscopy of 1–2 μm araldite sections revealed a highly refractile deposit within the nodal gap.

The rhodizonate method is not well suited to electron microscopy since the crystals are large and tend to pull out of thin sections, but it could be seen in this material that the crystals were predominantly located within the nodal gap substance (Fig. 6). The barium ion precipitated as barium sulphate was found to have formed a dense amorphous precipitate within the nodal gap substance (Fig. 7), frequently extending, as in the example illustrated, as a thin layer along the external surface of the myelin

sheath within the Schwann cell cytoplasm. With more prolonged staining, or with higher concentrations of barium chloride, the myelin of the whole terminal loop region was also stained, producing an effect similar to that observed by Herbst (1965) with lead nitrate staining of nodes.

Cupric ion

The cupric ion bound at nodes of Ranvier was demonstrated by its conversion to Hatchett's brown following treatment with acidified potassium ferrocyanide (Langley & Landon, 1969). Light microscopy showed the pigment localized as a dark brown ring around the nodes of Ranvier, and electron microscopy demonstrated that this ring was composed of an amorphous or finely granular precipitate restricted to the gap substance, with a few coarser crystals lying in the immediately adjacent endoneurium (Figs. 8, 9).

Lanthanum ion

This tervalent cation, although not used in the ion exchange experiments, showed a pattern of localization very similar to those of the ions so far described. Phase-contrast examination of 1·5 μm araldite sections of nerves which had been fixed in fixative solution containing 1 % lanthanum nitrate showed a highly refractile ring around the nodal axon. Electron microscopy showed this to be caused by a dense precipitate, often finely granular in part, localized within the gap substance, and extending in most instances as a lake beneath the terminal loops and within the intraperiod line of the external three or four lamellae of the myelin sheath (Figs. 10, 11).

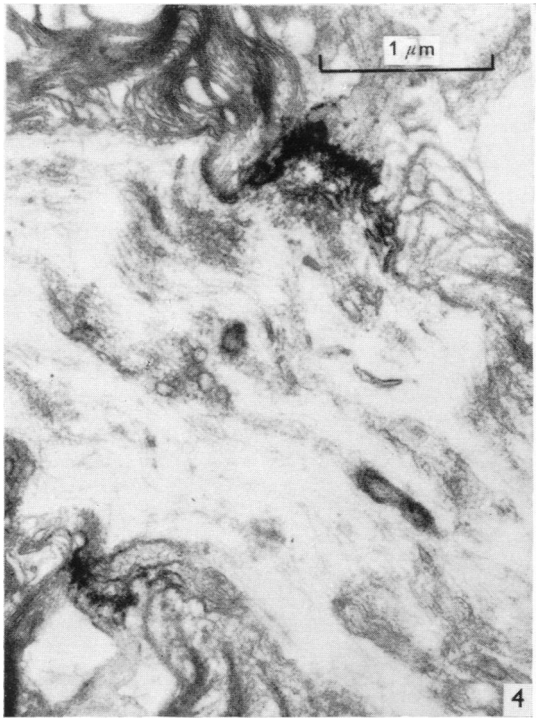
Potassium and sodium ions

Some of the ions used in ion exchange experiments (Langley, 1969; Landon & Langley, 1969) unfortunately proved not to be susceptible to fine structural identification. Potassium ion binding at nodes of Ranvier can be demonstrated for light microscopy by immersion of fixed or unfixed nerves in 0·1 M potassium chloride solution followed by the sodium cobaltinitrite reagent (Macallum, 1905). A yellow crystalline precipitate is formed within the area at the nodes and this can be converted into a black sulphide (Fig. 12) by treatment with ammonium sulphide. However, the crystals are invariably large, giving poor localization, and rendering the method unsuitable for electron microscopy.

The fine structural localization of sodium ion at the nodes of peripheral nerve fibres has also proved unsuccessful in our hands. The precipitation of sodium ion as sodium pyroantimonate by the method of Komnick (1962) was attempted, but the electron-dense precipitate produced was small in quantity and variable in location, a finding which confirms the observations of workers who have used this method on other tissues (Lane & Martin, 1969).

Enzyme digestion and chemical blockade

The site of the material binding those ions whose fine structural location could be accurately determined, i.e. ferric, cupric, barium and lanthanum ions, differed in several respects from that of the material stained by the Hale colloidal iron method. Whereas the latter appeared to be restricted to the superficial axoplasm of the node and the neighbouring innermost portion of the gap substance, the exchangeable cations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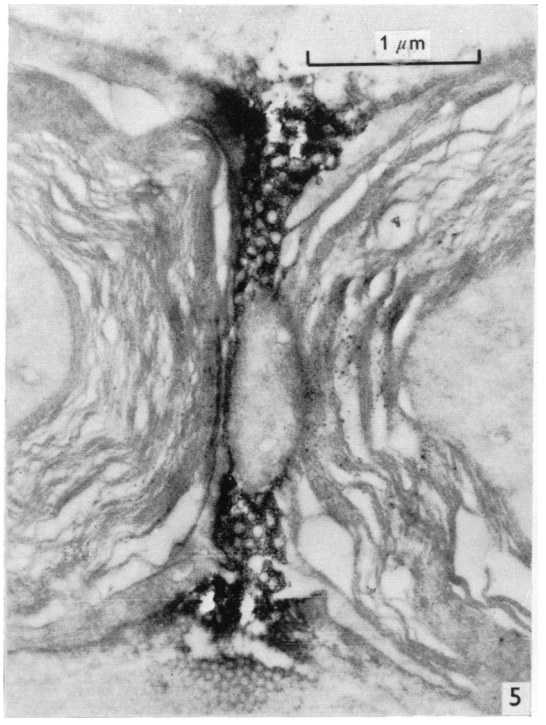
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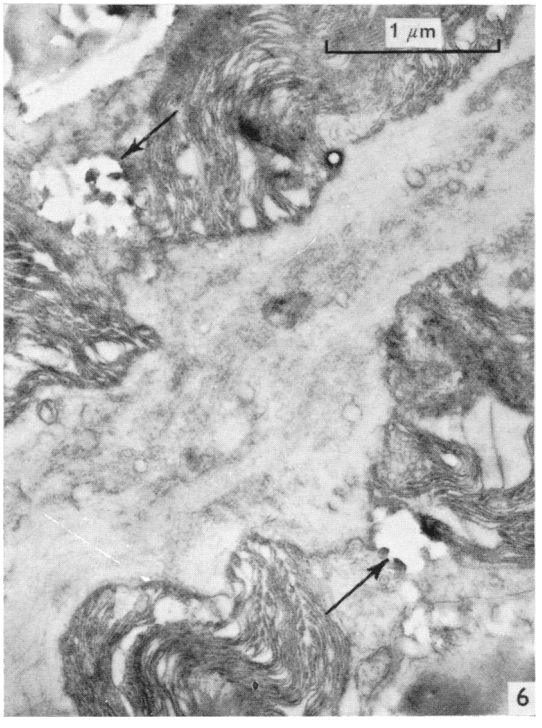
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were found throughout the gap substance, in the immediately adjacent endoneurium, beneath the outermost myelin loops and within the intraperiod line of the outermost lamellae of the myelin sheath. These differences suggested that other polyanions, unstained by the Hale method, were responsible for the greater part of the ion binding effect. Experiments in which the nodal binding material was subjected to enzymic digestion and chemical blockade provided some support for this suggestion. The Hale staining material is susceptible to digestion by testicular hyaluronidase, resistant to trypsin and irreversibly blocked by methylation and subsequent saponification (Langley & Landon, 1967). In contrast, the greater part of the ion binding material was unaffected by testicular hyaluronidase but readily digested by trypsin; its ion binding capacity was lost on methanol dehydration, indicating either irreversible loss or denaturation. Furthermore, the lowest pH at which this material would evince histochemical staining, pH 2·6 (Langley, 1969) was appreciably higher than that at which the Hale staining component would bind colloidal iron (pH 1·7). Neither component was affected by the actions of neuraminidase or phospholipase C.

Alcian blue

Additional support for a distinction between the Hale-stained and the other nodal ion binding materials was provided by the results of experiments using the Alcian blue staining technique of Scott (1968). The nodes of rat sciatic nerve fibres readily stained with Alcian blue at pH 5·7 to yield a transverse blue bar across the node (Fig. 13). However, the critical electrolyte concentration (C.E.C.), i.e. the concentration of electrolyte necessary to inhibit staining at that pH, was not sharply defined, but lay within a range of concentrations somewhat above the C.E.C. value obtained for Schwann cell nuclei, and well below that obtained for mast cell granules in the same material. These findings appear to indicate the presence of a mixture of polyanions at the site of nodal staining. A more extensive account of these and other experiments with Alcian blue will be published elsewhere (Langley, 1970).

Ruthenium red

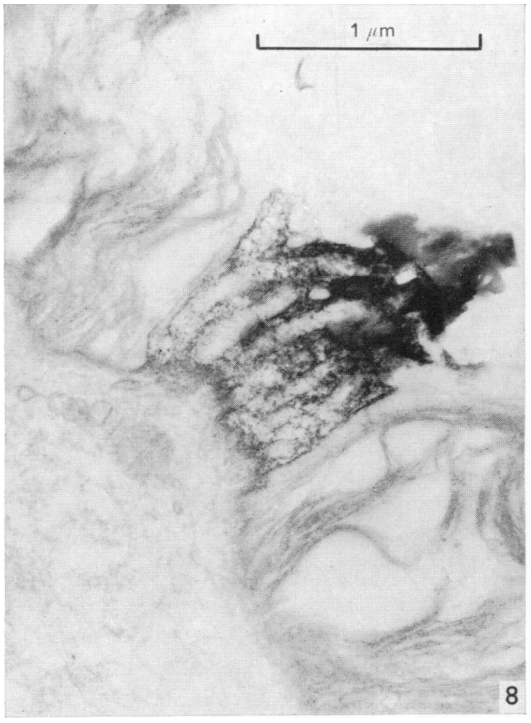
Whether nodal staining was effected by the addition of ruthenium red to the primary fixative, or by immersion in aqueous ruthenium red after fixation, an electron dense deposit was obtained, the distribution of which was peculiar to this particular stain and quite unlike the pattern of staining obtained with the exchangeable ions described above. This electron-dense deposit took the form of flakes or

Fig. 4. A longitudinal section of a node stained with the Hale colloidal iron method. A dense precipitate spans the nodal axolemma.

Fig. 5. An off-axis longitudinal section of the node of a fibre treated with ferric chloride followed by potassium ferrocyanide. A dense precipitate fills the gap substance revealing the microvilli of the Schwann cell in negative contrast. A more obviously crystalline precipitate forms an outer ring around the node in the endoneurium outside the basement membrane.

Fig. 6. Barium ion bound at a node demonstrated by its precipitation as barium rhodizonate. The crystals have been lost from the section but occupied the gap substance and areas in the adjacent endoneurium (arrows).

Fig. 7. A node at which the bound barium ion has been precipitated as its insoluble sulphate. Predominantly located in the gap substance, a thin layer of precipitate extends along the outermost lamellae of the myelin sheath (arrows).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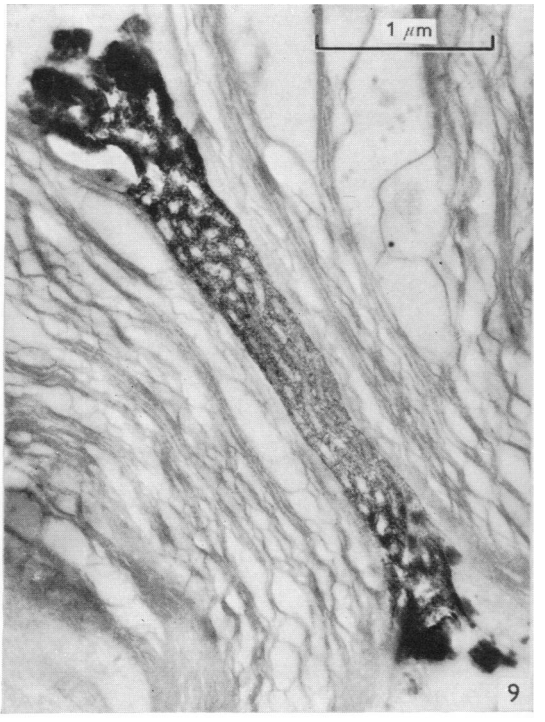
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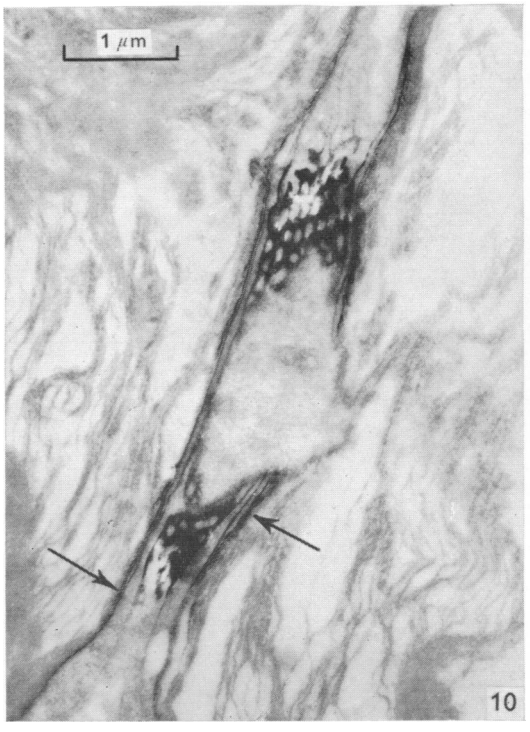
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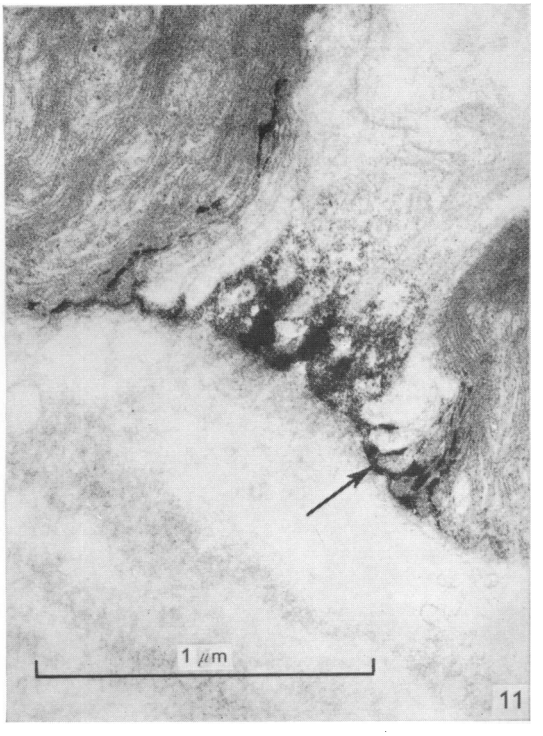
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particles attached to, or forming an integral part of, the plasma membranes of the axon and Schwann cell, including its microvillous nodal projections, and there was similar staining of the membranes forming the walls of such intracellular organelles as mitochondria. The gap substance contained only a pale flocculent precipitate and a similar pale deposit filled the normally clear zone between the Schwann cell plasma membrane and its basement membrane (Figs. 14, 15).

DISCUSSION

Our studies of ion exchange at the node of Ranvier (Landon & Langley, 1969; Langley, 1969) demonstrated the presence of a fixed anionic charged matrix surrounding the axon at the node, and within the incisures of Schmidt–Lantermann, and showed that the strength of the bond between the bound cation and its binding site increased with valency and was inversely proportional to hydrated ionic radius. These properties, together with the reversibility of the reaction, suggested that at this site, as in other experimental systems involving polyanions and low concentration of electrolyte (Bungenberg de Jong, 1949), the cation–polyanion bond is an electrostatic one.

The results reported in the present paper indicate that the fine structural location of all the exchangeable ions, except ruthenium, which were demonstrable by electron microscopy was the gap substance, although some ions did in addition stain other nodal and paranodal structures. While the sulphated material close to the nodal axolemma must be expected to play a part in the ion binding reaction, the fine structural location of the ion binding agent, and its response to blocking reactions, enzyme attack and Alcian blue staining indicate that other polyanions, unstained by the Hale method, are the cause of the majority of the observed cation binding effect. We suggest that polyanions containing carboxyl groups, or possibly a mixture containing carboxyl and phosphate groups with carboxylated polyanions predominating, are present in the region of the gap substance at the node of Ranvier, and that these polyanions are responsible for the greater part of the cation binding which we have demonstrated.

The staining pattern produced by ruthenium red differs from that of the other ionic stains in showing a marked affinity for both plasma membranes and the membranes of intracellular organelles. Ruthenium red is said to exist in solution as a polynuclear ionic coordination complex bearing a net positive charge of 6 (Fletcher *et al*, 1961). It has been used as a botanical stain for pectin, a carboxylated macromolecule (Jensen, 1962), and more recently as a stain for other negatively charged molecules in tissues of animal origin (Luft, 1966). It stains the erythrocyte surface as

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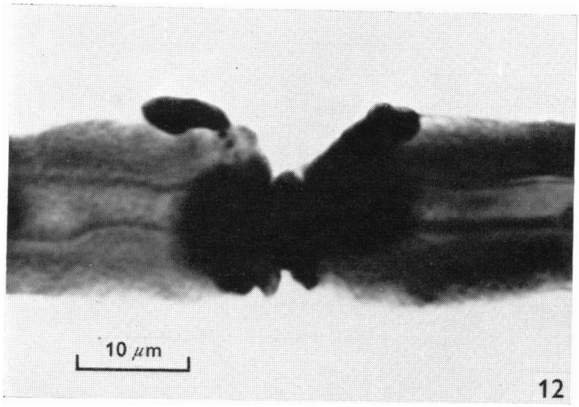
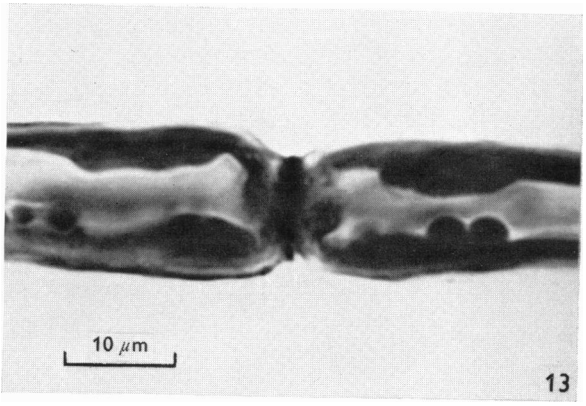
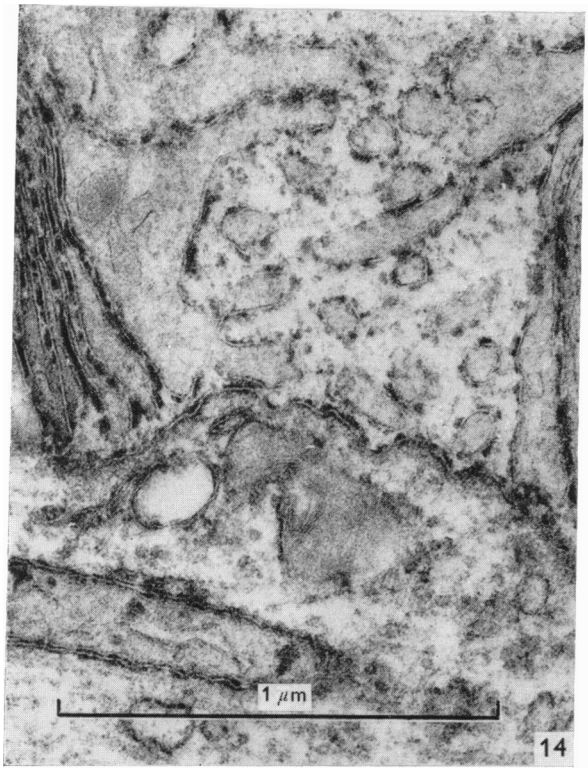
Figs 8, 9. Cupric ion bound at the node precipitated as Hatchett's brown by potassium ferrocyanide. Axial and off-axis longitudinal sections showing the finely granular precipitate throughout the gap substance and the coarse crystals in the immediately adjacent endoneurium. The Schwann cell microvilli stand out in negative contrast.

Figs 10, 11. Very similar appearances seen at the nodes of fibres treated with lanthanum nitrate. A consistent feature of staining with the lanthanum ion is its presence as a lake beneath the outer terminal loops and along the intraperiod line of the outermost lamellae of the myelin sheath (arrows).







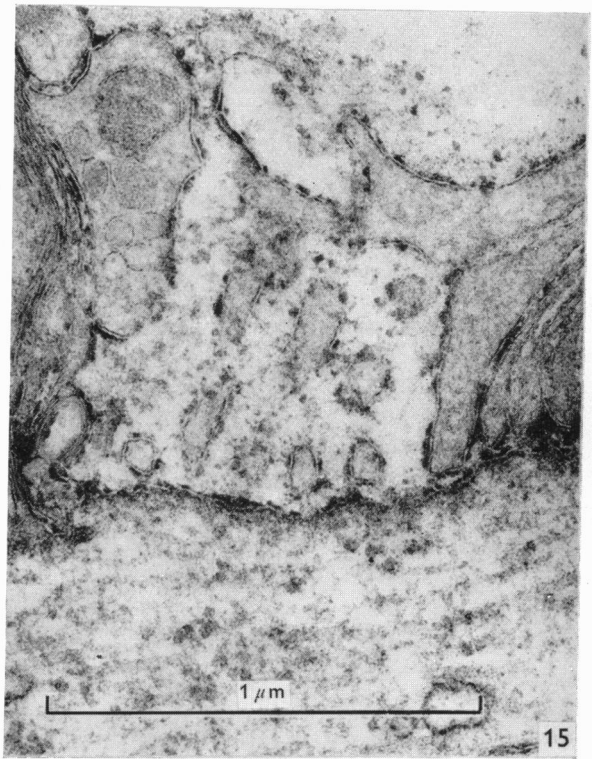


Fig. 12. The localization of potassium ion bound at the node of a teased osmicated nerve fibre demonstrated by the sodium cobaltinitrite technique. A dense precipitate of cobalt sulphide fills the nodal gap and extends into the juxtanodal myelin sheath.

Fig. 13. A teased osmicated nerve fibre stained with 0·01 % Alcian blue in 0·85 % saline for 2 h. The nodal gap is seen to be densely stained.

Figs 14, 15. The two sides of a longitudinally sectioned node stained with ruthenium red. Note the dense plaques associated with the external surface of the plasma membranes and the absence of a lucent interval between the plasma membrane and basement membrane of the Schwann cell.

a thin layer (Behnke, 1969) in which sialic acid is the predominant anionic group (Eylar, Madoff, Brodey & Oncley, 1962), and also the luminal surface of capillary endothelial cells (Luft, 1966), another site where sialic acid has been shown to be present (Gasic & Gasic, 1962; Rambourg, Neutra & Leblond, 1966). The surfaces of platelets, which possess sulphated mucopolysaccharides in addition to sialyl groups (Behnke, 1969), show a much greater reactivity to ruthenium red than do those of erythrocytes. Behnke concluded that ruthenium red stained carboxyl groups in the platelet surface and presumed that in part they were those of sialoproteins.

A considerable quantity of protein bound sialic acid can be extracted from rat peripheral myelinated nerve (Langley, in preparation) and calculations regarding the surface density of sialic acid molecules on the various membrane systems that exist in peripheral nerve suggest that sialic acid is absent from myelin and is confined to the axon and Schwann cell plasma membranes. It is possible that in our preparations the ruthenium red reaction product which coats the Schwann cell plasma membrane, and fills the otherwise electron-lucent gap between the plasma membrane and the basement membrane, demonstrates sites of negatively charged sialyl groups.

The application of these results to the physiological situation at the node *in vivo* obviously requires caution since the majority have been obtained from fixed material. Nevertheless, we consider that any attempt to analyse the mechanisms involved in the saltatory transmission of impulses along peripheral myelinated nerve fibres will require a detailed analysis of the possible effects of the presence of such a polyanion matrix around the node upon the ionic movements which occur at this site during the passage of the action potential.

Although we have used the term 'binding' without qualification to describe the condition of attachment to the polyanion of all the cationic species discussed, it is necessary to distinguish between the freedom of movement of, for example, a ferric ion and a sodium ion in this situation. Monovalent cations, such as the sodium ion, with a large hydrated ionic radius, whilst being bound in the sense of satisfying the condition of electroneutrality in the immediate neighbourhood of the polyanion, can probably move with relative freedom within the polyanion atmosphere and will be readily exchangeable for other ions which may be added to the matrix. On the other hand smaller, highly charged ions, such as the ferric ion, with strong polarizing effects, are likely to move much less freely within the polyanion field.

The polyanionic material we have demonstrated to be present at nodes may be capable of maintaining a relatively high concentration of sodium ion close to the nodal axolemma, and could thus act as a reservoir of sodium ion available for transit across the axolemma during the passage of the action potential. The release of sodium ion from this reservoir may be triggered, or encouraged, by the arrival within the matrix of calcium ion withdrawn catelectrotonically from the membrane phase of the axon (Tobias, 1964). Furthermore, the presence of such a polyanion matrix might limit the diffusion of the potassium ion which leaves the axon during the passage of the impulse and might hold it available for redistribution across the nodal axolemma during its return to the active state, whether by a fixed charged system (Ling, 1962) or an energy-consuming sodium pump (Judah & Ahmed, 1964). Bennett (1963) has proposed that the 'glycocalyx' of striated muscle may have a similar function in limiting the loss of potassium ion from the immediate vicinity of the sarcolemma

following its outward movement through the membrane during the passage of the propagated action potential.

A further possible function for the Schwann cell–gap substance complex may be postulated from an extension of certain earlier speculations regarding the significance of the large aggregations of mitochondria in the columns of Schwann cell cytoplasm immediately adjacent to the nodes of large myelinated fibres.

Landon & Williams (1963) proposed that the satellite cell functions of the Schwann cell might extend to the provision of energy-rich compounds to the nodal axolemma, whilst Berthold & Skoglund (1967) suggested that the paranodal mitochondria might play a specific role in the maintenance of the ionic milieu in the nodal region. One of us (D. N. L.) has been impressed for some time by the similarity of certain features of the nodal and paranodal portion of the Schwann cell to the cells of the salt secreting glands in many marine vertebrates; such cells, as well as those of other tissues known to be concerned in the active transcellular transport of ions, possess several characteristic cytological features. These include a plentiful supply of mitochondria close to their absorptive basal and lateral surfaces, and microvilli on their small apical secretory surface, which may project into a cap of sulphated mucopolysaccharide (Abel & Ellis, 1966; Bulger, 1963). Is it possible that the Schwann cell has a role to play in the active accumulation of sodium ion in the region of the nodal gap, the mitochondria of the paranode providing the energy for the active translocation of sodium ion from the endoneurial surface of the Schwann cell, or from its intramyelin compartment via the Schmidt–Lantermann incisures, to the cell's microvillous apex at the node?

A system of this kind might well be of little value to small diameter fibres which conduct relatively slowly, but could offer some advantage in large diameter fibres normally conducting prolonged trains of high-frequency impulses; it is in just such fibres that the paranodal apparatus and aggregations of Schwann cell mitochondria reach their greatest development. Observations which may be relevant to this proposal include those of Hodgkin (1951), that small changes in sodium-ion concentration external to nerve membranes, whilst having very little effect on the resting potential, can greatly alter the magnitude and rate of rise of the action potential. Again, Berthold & Skoglund (1967), showed that in growing feline nerves the appearance of paranodal mitochondrial aggregations corresponds well with the attainment of adult electrical properties with regard to absolute refractory period; and Skoglund (1960) found an increasing ability of growing nerve fibres to convey sustained discharges from muscle spindles – discharges which increased in frequency with increasing fibre size. In addition, Schwieler (1968) has shown in the newborn kitten that, although the bronchial and sinus nerves are composed of fibres of the same size as, or smaller than, those of the sural nerve, they are significantly more mature, both in their ability to conduct continuously at high frequencies, and in their pattern of NADH – tetrazolium reductase activity at the nodes. This suggests that nodal maturity, including the aggregation of mitochondria at the paranodes, may be a more important factor in determining the electrical properties of developing peripheral nerve fibres than their absolute diameter.

Hess & Young (1952) remarked that 'much remains obscure about the nature of the barrier around the fibre at the node'. This paper, and its predecessors (Langley &

Landon, 1967; Landon & Langley, 1969; Langley, 1969, 1970) represent an attempt to lessen this obscurity, but Hess & Young's further comment, that 'until the permeability properties of the cementing disc, perinodal space and outer endoneurium are known it cannot be assumed that at the node there is free diffusion between the axon surface and whatever tissue fluids constitute the external environment of the fibre', remains valid today. Whether the Schwann cell at the node is responsible for the active concentration of sodium ion in that region or whether it is merely concerned with the manufacture of a passive ion-binding matrix, the gap substance, it is certain that the functional behaviour of the nodal axon membrane cannot be considered in isolation from its natural morphological environment, and that the properties of the node of Ranvier derived from electrophysiological studies are the properties of the node in its entirety and not merely of its nodal axolemma.

SUMMARY

Experiments have been performed to determine the fine structural localization and chemical nature of the fixed anionic sites which bind cations at the nodes of Ranvier. The nodal gap substance was found to contain the majority of the bound cation and this material is considered to be composed predominantly of protein-linked carboxylated mucopolysaccharide. It is argued that the presence of such an ion-binding matrix surrounding the nodal axolemma could significantly affect the nature and magnitude of the ionic movements which accompany the passage of the nerve impulse, and it is suggested that the paranodal apparatus of the Schwann cell may play an active part in the accumulation of sodium ion within this nodal anionic matrix.

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