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THE MOVEMENT OF OPTICALLY DETECTABLE ORGANELLES IN MYELINATED AXONS OF *XENOPUS LAEVIS*

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SUMMARY

1. Intra-axonal organelles were detected by darkfield and Nomarski microscopy in isolated myelinated nerve fibres from *Xenopus laevis*. Nerve fibres from the 8th spinal roots, the sciatic nerve, and identified motor and sensory axons from other hind limb nerves were used. The movement of the organelles was recorded either on motion picture film or by noting the times at which they crossed the lines of an ocular grid.

2. Three groups of organelles were detected in all fibres. A group of particles with round profiles 0·2–0·5 μm in diameter moved somatopetally. Another group of round particles moved somatofugally. The ratio of the number of somatopetally travelling particles to the number of somatofugally travelling particles was about 10:1. The third group of organelles consisted of rod-shaped bodies about 0·2–0·3 μm in diameter and 1–8 μm in length; these were usually stationary.

3. All the round particles appeared to move independently of each other with a saltatory motion. The somatopetally and somatofugally travelling particles had statistically different mean velocities of 0·98 and 1·32 μm/sec respectively.

4. Round particles often crossed the node of Ranvier with no appreciable change in velocity. Some, however, were temporarily arrested at the entrance to the node.

5. While the rod-shaped organelles were usually stationary, they occasionally moved rapidly lengthwise for distances of up to 10 μm. Rarely a rod-shaped organelle exhibited a continuous saltatory motion.

6. Round particles often travelled in either direction along the edge of rod-shaped organelles. One rod was observed to move along the path previously taken by a round particle.

7. The findings are discussed with respect to (*a*) the normality of the preparations, (*b*) the numbers of particles travelling in each direction,

(*c*) the nature of the organelles, and (*d*) the mechanisms underlying the motion.

8. We suggest that particles move along microtubules which have specific directionalities and particle affinities. The microtubules are in bundles and are closely associated with rod-shaped mitochondria.

INTRODUCTION

The transport of materials along nerve fibres in mature animals has been demonstrated most clearly by labelling proteins with radioactive tracer elements (Lasek, 1970; Ochs, 1970; Dahlström, 1971). This and other biochemical techniques are applied successfully only to large numbers of neurones whose axons run together for appreciable distances. In general, such studies have demonstrated somatofugal movements of materials at a slow rate (about 1 mm/day) and at a fast rate (100–1000 mm/day). Transport of material towards the soma is, for technical reasons, less well described and is thought to take place at intermediate rates (10–100 mm/day).

Light microscopy of tissue cultured neurones has shown that many intra-axonal organelles are in motion both towards and away from the cell body (Matsumoto, 1920; Hughes, 1953; Nakai, 1956; Pomerat, Hendelman, Raiborn & Massey, 1967). Some of these organelles may move at velocities which are equivalent to the rates of fast axoplasmic transport described in adult tissue (Burdwood, 1965). However, the relationship between these observations and axoplasmic transport in adult nerve has been difficult to assess; in fact, it has been denied that there is any relationship between the phenomena (Weiss, 1967).

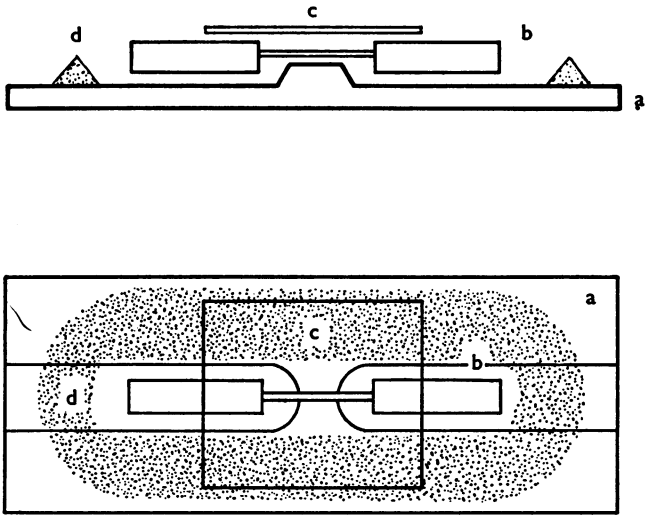
Recently there have been reports of the optical detection of rapidly moving organelles in nerve fibres isolated from adult vertebrates (Smith, 1971, 1972*a*, 1973; Kirkpatrick, Bray & Palmer, 1972; Kirkpatrick & Stern, 1973). Hence, it is now possible to examine the mechanisms and significance of the transport of large organelles in single neurones of adult animals. The findings can be related to those from embryonic or cultured tissue and to the view of axoplasmic transport in adult nerve as obtained by other techniques.

This paper describes the general features of the motion of optically detectable organelles in myelinated nerve fibres isolated from adult *Xenopus laevis*.

METHODS

The animals used were adult male and female *Xenopus laevis* (African clawed toad). These animals were in good nutritional condition and were kept in tanks of water at room temperature. In most experiments the animals were pithed, but in

some cases, as for the isolation of parts of the spinal root system, they were anaesthetized by submersion in a 2% solution of urethane (ethyl carbamate). Nerves were dissected free from the animal, and single myelinated nerve fibres were subsequently isolated, under a Ringer solution of composition (mM): NaCl, 112; $NaHCO_3$, 2·5; KCl, 2·5; $CaCl_2$, 2·0. The Ringer solution was gassed with 95% O_2 and 5% CO_2 before use. All experiments were conducted at room temperature (20–22° C).



Text-fig. 1. Diagrammatic top view, below, and section, above, of the viewing chamber. A microscope slide (a) had two longitudinal grooves 7 mm wide milled to half the thickness of the slide. At the centre of the slide the two grooves were separated by a table 2 mm wide which supported the single nerve fibre. The dissected piece of nerve is shown in place (b). The nerve was immersed in a Ringer solution (not shown). A cover glass (c) was placed over the preparation. A layer of petroleum jelly (d), indicated by the stipple, prevented the cover glass from touching the single nerve fibre and also sealed the cover glass to the slide. The petroleum jelly at the ends of the slide prevented the Ringer solution from leaking off the slide.

Most of the observations were on nerve fibres which were 10–20 μm in diameter. These were isolated at the centre of a 5 cm length of sciatic nerve taken from the hip to just below the knee joint. Approximately fifty such preparations were used. In a small number of cases fibres from parts of the 8th spinal roots and from identified sensory and motor nerves in the leg were used: ventral root fibres, three experiments; dorsal root fibres central to the dorsal root ganglion, five experiments; dorsal root fibres distal to the ganglion, three; sensory nerve fibres to the knee joint and to leg skin, three; large diameter motor nerve fibres to the iliofibularis muscle (identified as in Smith & Lännergren, 1968), four.

The isolated nerve fibres were mounted for observation in a chamber made from a microscope slide (Text-fig. 1). Two longitudinal grooves 7 mm wide were milled with a diamond cutter in each half of the slide to a depth of half the thickness of the slide. A central table about 2 mm wide separated the two grooves. The undissected portions of the nerve rested in each groove while the table supported the single nerve

fibre. The top surface of the slide was lightly greased with petroleum jelly to support and seal a cover-slip. This chamber allowed very close apposition of the cover-slip to the isolated part of the nerve fibre without crushing those parts remaining in the undissected nerve bundle. Fresh Ringer solution could be perfused through the chamber by dripping it into one end of the central groove and removing the solution emerging at the far side of the cover-slip.

Organelles within the axoplasm of the single nerve fibres could be detected using either Nomarski optics or darkfield optics (Smith, 1971, 1972*a*). In most cases darkfield illumination was the more useful. A Zeiss Ultra darkfield condenser was used with a 100× planachromatic objective at a numerical aperture of 0·9–0·8. The light source was a quartz-iodide lamp. Heat filters in the optical path prevented unintentional heating of the preparation.

The motion of organelles within the axoplasm was recorded either on motion picture film or by noting the times at which organelles crossed the fine lines of an ocular grid. Motion pictures of the darkfield image were taken at 3 or 4 frames/sec with Kodak 2479 RAR 16 mm film. The camera, mounted to a rigid frame separate from the microscope, was driven by a servomotor via a flexible coupling. Nomarski images could be filmed at 12 frames/sec with Kodak 4X film. The motion pictures were subsequently projected and measurements made using a frame-counting projector. Distances were calibrated against the image of a 10 μm grid. With the alternate method of recording organelle motion an ocular grid was used having 20 lines spaced either 5·0 μm or 3·13 μm apart. The lines were arranged to fall across the nerve fibre at right angles to its axis. Each time an organelle crossed one of the lines the observer closed a microswitch causing a line to be drawn across a strip chart. The time axis on the strip chart record was calibrated with a 1 Hz square wave. This method had the advantage that organelles could be followed for distances of up to 100 μm by adjusting the focus slightly. On the other hand, motion picture film in addition to providing a permanent record of typical events also captured rare events. The results of either of the methods allowed distance–time plots to be constructed for the motion of individual organelles. Straight lines were fitted to these plots by a least squares method. Except where noted in the text, sufficient data were gathered so that the correlation coefficient between the variables (distance and time) was at least 0·98. The slopes of the lines then gave mean velocities for individual organelles. The velocities so obtained represented transport velocities in the axial direction of the nerve fibre. Except in unusual cases this value was very close to the speed of the organelle along its trajectory; markedly curving trajectories through the axoplasm were not common.

RESULTS

General observations

Darkfield microscopy (Pl. 1) revealed in all axons, including the known sensory and motor fibres, three groups of organelles. Particles with round (diameter 0·2–0·5 μm) images or slightly elliptical images (0·2 to 0·3 × 0·5 μm) moved at about 1 μm/sec in the somatopetal, or, in the sciatic nerve, proximal, direction. A similarly shaped group of organelles with images 0·2–0·3 μm in diameter moved at a similar rapid rate in the opposite direction, away from the cell body. The motion of organelles in these two groups was irregular in a manner that is termed saltatory (Rebhun, 1972; Berlinrood, McGee-Russell & Allen, 1972). Each particle appeared to

move independently of the others and both streams of particles occupied the whole axoplasmic space within the internodes. While any given particle might hesitate in its course, and some even reverse direction for a few micrometres, no particle changed its direction of movement entirely. Two differences were noted between these two groups of organelles: the somatopetally moving group contained about 10 times as many detectable organelles as did the somatofugally moving group; the former group also contained the largest organelles.

The third group of organelles consisted of rod-shaped bodies approximately 0·2–0·3 μm in diameter with lengths ranging from 1 to 8 μm. These were usually stationary in the axoplasm although occasionally a rod was observed with a saltatory motion similar to that of the round organelles.

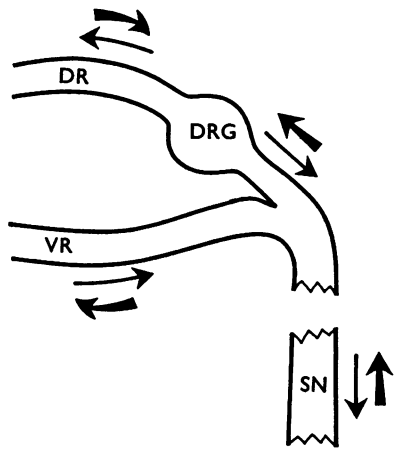
In the following parts of this report these observations will be treated more fully. A detailed analysis of the erratic motion of individual organelles will, however, not be undertaken here; consequently the use of the descriptive term saltatory requires some justification. The term is applied to the rapid (in the order of microns per second) and irregular, or jumping, motion of intracellular particles that is not likely to be a consequence of thermal agitation (Rebhun, 1972). In this work the discontinuous jumping motion was most obviously present in ageing preparations (see section on status of the preparations). However, this kind of jumping motion also occurred in fresh preparations, as shown in Text-fig. 8, open circles, and Text-fig. 9. Most of the round organelles in fresh preparations did not show such clear saltatory motion, in these the jumps were more closely linked giving rise to an almost continuous motion which took place in what appeared to be major periods of acceleration and retardation of about 10 μm length (Text-fig. 4, lower part). Thus it seemed that the motion of all the organelles observed in this study might reasonably be described as saltatory. Coarse sampling of the trajectories of particles did not, in general, reveal these fluctuations in velocity (see Text-fig. 5).

Status of the preparations

Most of the results were obtained from observation of the axoplasm at locations which were about 2·5 cm distant from the cut ends of the fibre. Effects of severing the axons on the motion of the organelles cannot be discounted; there obviously must be a marked effect close to the site of nerve section. It has not yet been possible to view these effects very close to the cut ends owing to the poor optical conditions there. In two cases organelle movements were observed 1 mm from the cut end between 20 and 35 min after the cut was made. The motion of these organelles did not differ in any detectable way from that usually observed at the centre of a 5 cm length of sciatic nerve. On the other hand, damage at or very close to the portion of nerve being observed was immediately apparent; the myelin became wrinkled

and particle motion in the longitudinal axis of the fibre ceased and was replaced by a Brownian motion.

The length of time that the preparations could be considered to be in a steady state varied from nerve to nerve. Two types of changes were characteristic of an ageing preparation: the number of organelles passing a given diameter of the axon per unit time decreased, and later the motion of individual organelles altered. For example, a typical preparation had a mean of 8·2 particles/min crossing a given diameter of the axon in one focal plane during the first hour of observation. At the end of the second hour the number was 8·4 particles/min. Thereafter the number of moving particles declined: 5·2/min at 3 hr and 1·6/min at 4 hr. Alterations in the motion of the particles became noticeable at later times; the pause between saltatory jumps became very long for a large number of particles. Normal motion of individual organelles was observed in some axons up to 14 hr after the nerve was removed from the animal. The measurements and observations given in the rest of this report were made during the first 2 hr that the preparations were in the form of steady state described above.

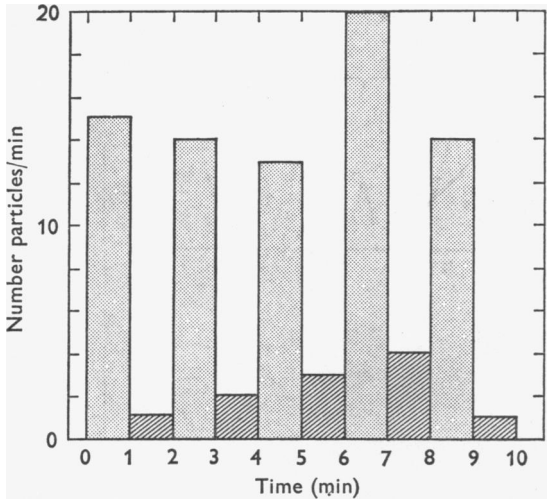


Text-fig. 2. Diagram of the 8th spinal roots and the sciatic nerve to show the directions in which most particles (thick arrows) and least particles (fine arrows) were seen to move. In all cases most particles travelled somatopetally. The ratio of the numbers of particles travelling in the two directions was at least 10:1 at all locations, but fewer moving particles were detected in the dorsal roots central to the dorsal root ganglion than at the other locations. DRG, dorsal root ganglion. DR, dorsal root. VR, ventral root. SN, sciatic nerve.

Motion of the round organelles

1. *Direction of motion.* The majority of round organelles detectable with either Nomarski or darkfield optics moved toward the soma of the nerve cell. In axons from the sciatic nerve, ventral roots, and dorsal roots distal to the dorsal root ganglion, about 10 times as many particles moved proximally (towards the spinal cord) as distally (Text-figs. 2 and 3). In axons from the dorsal roots central to the dorsal root ganglion most of the

particles moved away from the spinal cord and toward the ganglion (Text-fig. 2). Thus, the terms somatopetal and somatofugal, while cumbersome, are appropriate to describe the directions of movement of the larger and smaller groups of particles.

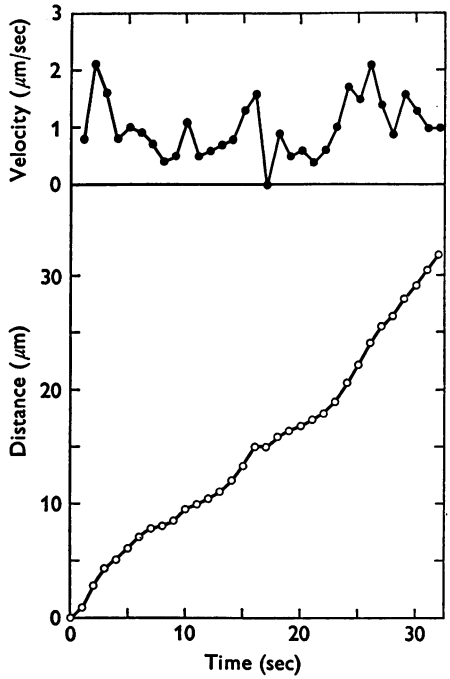


Text-fig. 3. Histogram showing the numbers of particles which crossed a diameter of the axis cylinder in the somatopetal direction (light bars) and in the somatofugal direction (dark bars) in alternate 1 min intervals. Diameter, 9·5 μm. Sciatic nerve fibre.

An analysis of darkfield motion pictures representing continuous periods of observation of 25 min for each of three sciatic nerve fibres gave a mean value for the number of particles crossing a diameter of the axis cylinder of 9·0 particles/min per 10 μm diameter in the somatopetal direction, and 0·57 particles/min per 10 μm diameter in the somatofugal direction (ratio 15·8:1). Fibres from the dorsal roots central to the dorsal root ganglion contained fewer moving particles. Motion pictures of two of these fibres gave mean values of 2·47 particles/min somatopetally and 0·25 particles/min somatofugally for 10 μm widths of axon. Here the ratio of somatopetally to somatofugally moving particles was close to 10:1.

2. *Velocities.* Text-fig. 4 shows in the lower graph the typical time course of displacement of a particle travelling in the somatopetal direction in a sciatic nerve fibre. The over-all motion was characteristically irregular, consisting of what are assumed to be closely linked saltatory jumps. The velocity of the particle (Text-fig. 4, top) was derived from the measurements of the lower part of Text-fig. 4 and was from moment to moment quite variable. We did not pursue the question of whether these small

scale variations in velocity were real or were caused by errors in measurement; the purpose of Text-fig. 4 is to demonstrate that the motion of the particle could be described in terms of a mean velocity of about 1 μm/sec. Distance–time plots for three representative particles travelling in the somatopetal direction are shown in Text-fig. 5*A*, and for the somatofugal direction in Text-fig. 5*B*. Each set of points is fitted with a least squares

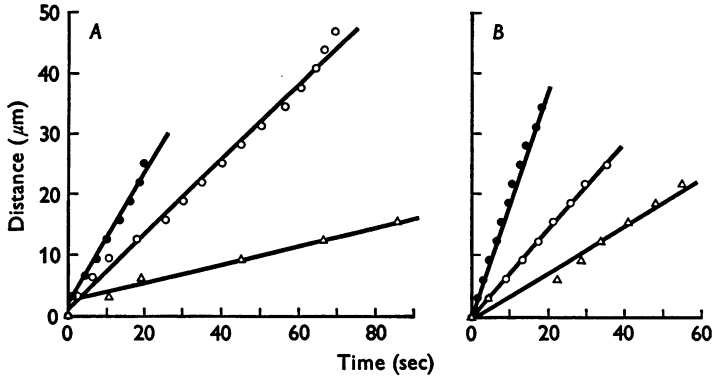


Text-fig. 4. The motion of a single particle travelling somatopetally. The lower part of the Figure is a plot of distance travelled against time. Velocity against time is plotted in the upper part of the Figure. The estimates of velocity were obtained from the data of the distance–time plot.

straight line whose slope represents the mean velocity of the particle. Text-fig. 6*A* is a histogram of 150 such mean velocities for somatopetally travelling particles. Text-fig. 6*C* is a similar set of values for fifty somatofugally travelling particles. The means and standard deviations for these two sets of determinations are $0{\cdot}98 \pm 0{\cdot}37$ μm/sec and $1{\cdot}32 \pm 0{\cdot}49$ μm/sec respectively. The mean velocities for the two groups are statistically different ($P < 0{\cdot}01$).

There was however some doubt as to whether these two groups of values could be fairly compared. The separate determinations of particle velocity were obtained from eight preparations by the ocular grid method. Analysis of the records

indicated that bias might have existed in the sampling of each preparation; on some occasions there was a tendency to record slower particles and on other occasions the faster particles were preferred. Consequently, an attempt was made to remove observer bias and to correct for the unequal weighting of samples by selecting randomly from each experiment a number of somatopetally travelling particles equal to the number of somatofugally travelling particles recorded in the same experiment. The distribution of the velocities of this subset of the somatopetally travelling particles is shown in Text-fig. 6 *B*; the mean and standard deviation of the distribution are $1{\cdot}05 \pm 0{\cdot}35$ μm/sec. The means of the distributions shown in Text-fig. 6 *A* and *B* are not statistically different, but the mean of the distribution of Text-fig. 6 *B* (somatopetally travelling particles) is still different from that of Text-fig. 6 *C* (somatofugally travelling particles) at the 5 % level of significance.

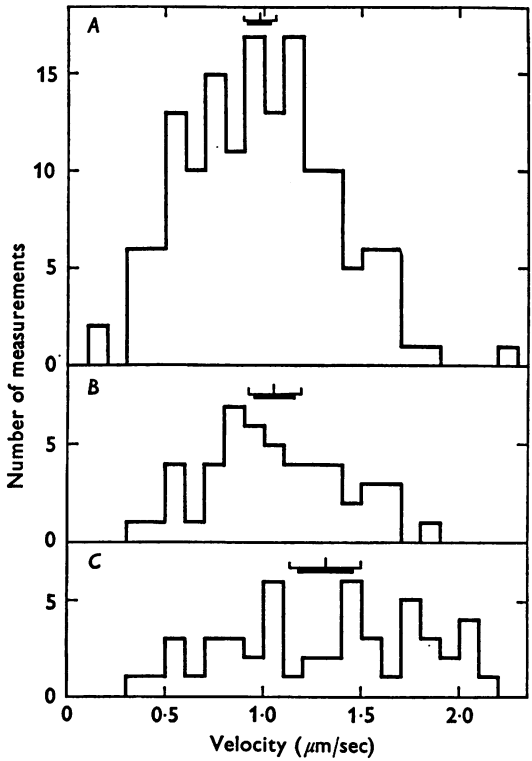


Text-fig. 5. Example distance–time plots for three particles travelling somatopetally (*A*) and three particles travelling somatofugally (*B*). Each set of values is fitted with a straight line whose slope describes the mean velocity of the particle.

Thus, from this set of data, one is led to the conclusion that the mean velocity of the somatofugally travelling particles was greater than that of the somatopetally travelling particles.

3. *Motion across nodes of Ranvier.* Measurements of the velocities of organelles in the internode of the myelinated nerve fibre can be extrapolated to long distances of the axon only if the organelles are not greatly impeded at the node of Ranvier. Observations of organelles at nodes of Ranvier were very difficult owing to the light scattered by the edges of the myelin. Observation by darkfield microscopy was successful once and four preparations were observed with Nomarski optics. Examples of trajectories followed by a number of organelles crossing the node in the somatopetal direction in one preparation are shown in Text-fig. 7. The envelope of the trajectories entering this node followed the shape of the nodal constriction but particles tended to remain centrally placed in the axoplasm on leaving the node. Some particles stopped on entering the

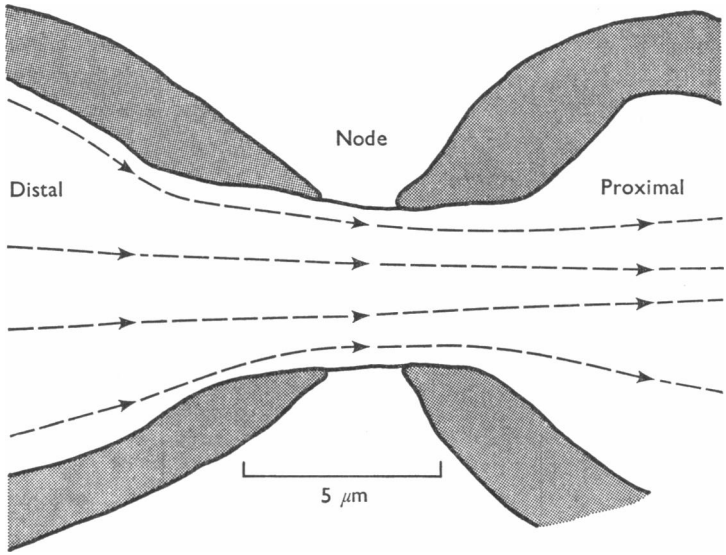
node (filled circles, Text-fig. 8) and continued their progress across the node after variable periods of time. A particle occasionally remained arrested at the inlet of a node for several minutes. Other particles (open circles, Text-fig. 8) traversed the node without any apparent hindrance. The two curves of Text-fig. 8 demonstrate that at the node, as elsewhere



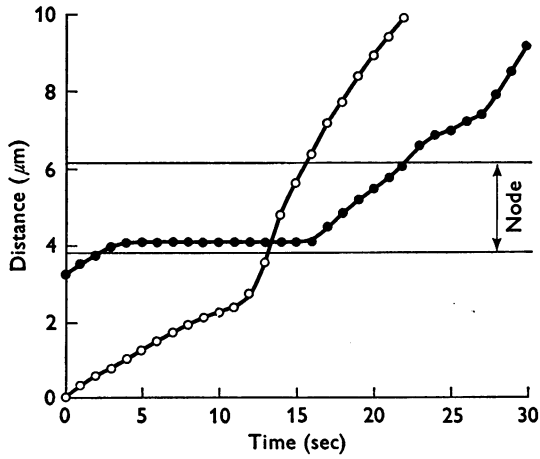
Text-fig. 6. Histograms of the mean velocities of individual particles in sciatic nerve fibres. *A*, a collection of 150 velocities of somatopetally travelling particles. *C*, 50 velocities of somatofugally travelling particles. *B*, a subset of the group of velocities shown in *A* chosen in such a way as to remove sampling bias (see small print in text); number in sample is 50. Above each distribution are indicated the position of the mean of the distribution and the 99 % confidence interval for the mean (fine lines) and the 95 % confidence interval for the mean (thick bar).

in the axon, the motion of any one particle appeared to be independent of that of its neighbours. The one successful experiment in observing the node by darkfield illumination showed that round organelles crossed the node somatofugally in a similar manner.

The hesitation of some organelles at the entrance to the nodal

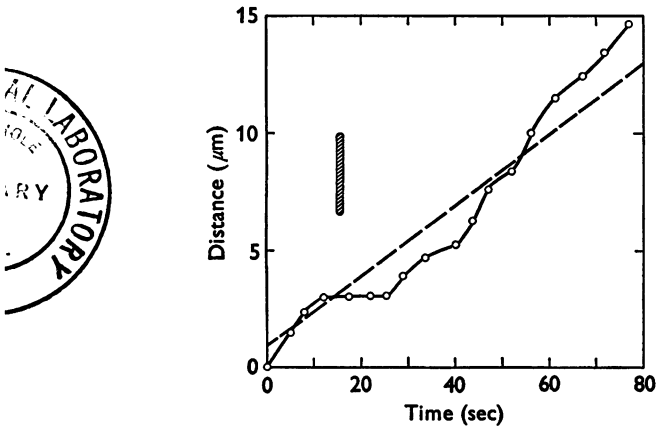


Text-fig. 7. A node of Ranvier drawn from motion picture film of a sciatic nerve fibre. The myelin is shaded. The proximal, right hand, side of the node is the side closest to the spinal cord. Trajectories of particles crossing the node in the somatopetal direction are shown; dashed lines and arrows. The outer two trajectories represent the envelope of fifty particle trajectories.



Text-fig. 8. The motion of two particles crossing a node of Ranvier in the somatopetal direction. Position of the node indicated by horizontal lines. Filled circles indicate the motion of a particle which hesitated for about 10 sec on entering the node. Open circles indicate the motion of a particle which was not impeded at the node. In the latter case the beginning of a saltatory jump occurred just before the node. Note that the motions of the two particles do not appear to be related.

constriction caused a local decrease in the mean velocity of organelles. A group of fifty organelles crossing in the somatopetal direction took an average of 20·2 sec to traverse a 10 μm distance spanning the node as compared to an expected 10 sec for a similar distance in the internode. This slowing, however, would not appreciably affect the transport rates over long stretches of axon.



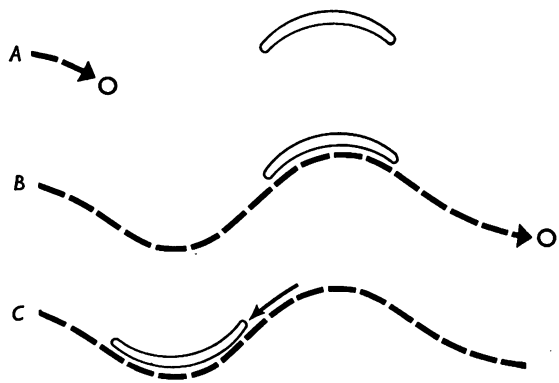
Text-fig. 9. Motion of a rod-shaped organelle in the somatofugal direction. Inset is a diagram of the organelle which was 0·3 μm wide and 3·5 μm long (scale as on the ordinate of the graph). The organelle moved in a series of saltatory jumps at a mean velocity (dashed line) of about 0·15 μm/sec.

Motion of the rod-shaped organelles

The majority of rod-shaped organelles remained stationary within the axoplasm. Extended periods of observation and motion picture films failed specifically to detect any movement at the slow axoplasmic transport rate (approximately 1 mm/24 hr or 40 μm/hr). Some rod-shaped organelles displayed a sudden axial shift in the axoplasm of up to 10 μm in either direction at velocities comparable to, or somewhat less than, those of most of the round organelles. These sudden shifts were only rarely followed by any further motion. More rarely yet, rods were seen moving with a continuous saltatory motion. Text-fig. 9 shows the time course of displacement in the somatofugal direction of an organelle which was 0·3 μm wide and 3·5 μm long. Its course over 15 μm was taken from motion picture film. The dashed line of Text-fig. 9 has a slope of 0·15 μm/sec and represents the velocity of the organelle over a 45 μm path in which the details of motion could not be entirely observed since the organelle moved out of and back into the focal plane.

Relationship between the motion of round organelles and the rods

A very evident phenomenon was the tendency of the round organelles to run along the edge of stationary rod-shaped organelles. Particles travelling in both directions took paths alongside the same rod. No other preferred pathways were noted. A particularly interesting sequence was observed in a sciatic nerve fibre in which, at one location, the rod-shaped organelles were arranged in a waved, roughly sinusoidal, pattern. There was no evidence that this axon was damaged in any way. Round organelles entering this region in either direction followed axoplasmic pathways matching the arrangement of the rods. Text-fig. 10*A* diagrams the beginning of a sequence in which a round organelle travelling somatopetally



Text-fig. 10. A diagram of a sequence in which a somatopetally travelling particle entered an area of folded axoplasm (*A*) and proceeded to travel along the edge of a rod-shaped organelle (*B*). A few seconds after the passage of the particle the rod moved along the path previously taken by the particle to a new stationary position as in *C*.

entered this region. Its curving pathway through the axoplasm included the edge of a rod-shaped organelle (Text-fig. 10*B*). Within a second or two of the passage of the round organelle past the rod, the rod moved rapidly in the somatofugal direction. The pathway taken by the rod and the final position it assumed matched exactly the pathway previously taken by the round organelle (Text-fig. 10*C*). These observations indicate that there is a relationship between the positions occupied by, and the movement pathways of, rod-shaped organelles and the pathways followed by round organelles. The simplest explanation would be that some structure, or axoplasmic track, associated with the mechanism of organelle movement runs along the edge of rod-shaped organelles.

DISCUSSION

We will discuss the results of this paper by considering four questions. Since none of these questions can be answered with any certainty at this time, each may suggest directions for future work.

Is this a description of a normal process?

Since the preparations consisted of lengths of nerve isolated from the animal, it is not certain that the results represent the behaviour of organelles in intact nerve cells. However, several lines of evidence do suggest that the condition of the nerve fibres used in these experiments was not greatly different from that of intact cells.

Most of the observations were made at distances of the order of 1000 fibre diameters from either cut end of the preparation. Fibres from the same animal are known to remain electrically intact for many hours at such distances from the cut ends (e.g. Hutchinson, Koles & Smith, 1970); presumably this reflects a normal ionic milieu within the axoplasm. Close to the cut ends conditions must be abnormal but our work indicates that the abnormal zone is likely to lie within the terminal few millimetres of the preparation during the period when measurements were being made. It has been shown (Lubinska, 1956; Johnson, Smith & Lock, 1969) that axoplasm is generally not lost from the cut ends of the nerve, although axoplasm is transferred from the terminal few hundred microns of nerve to a myelin-covered ball at the end of the nerve fibre. Both the rate of movement of the terminal axoplasm and the amount shifted are small, hence it did not seem likely that this effect would appreciably influence our measurements. Bulk transfer of axoplasm toward either cut end would, had it existed, have been detected in the motion pictures.

Saltatory particle movement in intact tissue cultured neurones of *Xenopus laevis* takes place at maximal ('instantaneous') velocities ranging from 0·6–2·25 μm/sec (Berlinrood *et al.* 1972). Edström & Mattsson (1972) give 90 mm/day (1·0 μm/sec) for the fast somatofugal movement of radioactively labelled proteins in relatively intact frog neurones, while 60 mm/day is reported for the somatopetal transport of similar radioactive labels (Edstrom & Hanson, 1973). Indirect measurements of the rates of movement of various enzymes in isolated segments of frog sciatic nerve (Partlow, Ross, Motwani & McDougal, 1972) give values varying between 11 and 99 mm/day (0·13 and 1·1 μm/sec), depending on the enzyme assayed and the direction of movement. The latter experiments were carried out over very long periods, up to 96 hr. The rates of particle movement obtained in our work are very close to those given in the work cited above. In addition, Ochs & Ranish (1970) have produced evidence that

fast exoplasmic transport in mammalian neurones is not dependent on the integrity of the whole nerve cell. If, however, future work should show that observations on isolated segments of nerve are not at all representative of normal cell behaviour, then this itself would be of great significance for the understanding of degeneration in neurones.

Do more organelles move in one direction than in the other?

Kirkpatrick and associates have recently reported particle movement in myelinated axons from chickens and from humans (Kirkpatrick *et al.* 1972; Kirkpatrick & Stern, 1973). In general the phenomena reported by these workers are very similar to those reported here. Spherical particles of about 0·2–0·5 μm diameter moved both somatopetally and somatofugally. The velocity of these in chicken nerve was given as 1·24 $\pm$ 0·48 μm/sec (mean $\pm$ S.D., $n = 46$, temperature 31° C). Rod-shaped organelles were also present in the axoplasm and these were occasionally seen to move. There are, however, some significant differences between the results of these workers and ours. Both in chicken and human nerve the majority of round organelles moved in the somatofugal direction rather than the somatopetal direction as described here. This difference is not likely to be due to some unique characteristic of amphibian nerve since in work on rat nerve (Smith, 1972*a*) the majority of organelles also moved somatopetally. The report by Edström & Hanson (1973) that in frog nerve the amount of radioactively labelled protein transported in the somatopetal direction is less than 10% of that transported in the somatofugal direction is probably not pertinent to the issue. This latter finding is more likely to reflect the relatively small amount of protein synthesized at nerve terminals than the amount of material being moved.

The problem presented by these different reports will be resolved only through further work. In the meantime, however, the following remarks may be pertinent. That a given optical technique detects more particles moving in the one direction than in another does not necessarily mean that more material is being transported in that direction. The threshold for optical detection by either of the techniques used (Nomarski or darkfield optics) depends largely on the difference in the refractive indices between the object (an organelle) and its surrounds, and on geometrical factors such as the thickness of the object. The diameter threshold for detectability of the organelles in nerve has been estimated at about 0·2 μm for darkfield optics (Smith, 1972*a*) and it is probably about the same for Nomarski optics used under the most favourable conditions. It follows that if one group of organelles, those moving somatofugally in our experiments, contains many organelles with a size less than the threshold for detection and if another group, those moving somatopetally,

contains organelles whose size is generally greater than the detection threshold, then more organelles will be observed moving somatopetally although the two groups may contain the same numbers of organelles. A similar argument could be used with respect to the density or refractive index of the organelles. Our results are understandable if one views the process observed in the axon as part of a circulation of organelles which originate at the soma and increase in diameter or density as they mature and finally return toward the soma. No doubt an hypothesis which is the inverse of this would explain the results of Kirkpatrick and associates. The remaining possibility, which still does not rule out the influence of optical detectability, is that in *Xenopus* there are in fact more organelles moving somatopetally than somatofugally. Observations on the uptake and somatopetal transport of horseradish peroxidase by axons would lend support to this idea (e.g. Litchy, 1973).

What are the organelles?

The paper by Matsumoto in 1920 is remarkable in that at that early date he identified, by means of vital staining, several classes of intra-axonal organelles in tissue-cultured sympathetic neurones. He detected rod-shaped and granular mitochondria by staining with Janus black, and described a group of granules and some vacuoles that stained with neutral red. The mitochondria showed a 'moderate degree' of movement while the granules which took up neutral red showed 'considerable movement, especially in a longitudinal direction'. In recent years it has been assumed by most workers that the optically detectable rod-shaped organelles in axons are mitochondria. This assumption is well supported by the long, axially oriented, profiles of mitochondria that are seen by electron-microscopy in thin sections of axons, and by the studies of mitochondrial shapes in some vertebrate nerves (Blume & Scharf, 1964). The nature of the round organelles is much more uncertain. From Matsumoto's work one can assume that in embryonic sympathetic nerves this group is composed of mitochondria (granules which stain with Janus black), and granules and vacuoles which stain with neutral red. It has been suggested that a large proportion of the round organelles seen in motion in adult *Xenopus* axons are mitochondria (Smith,1971, 1972*a*, *b*, 1973), however, the full evidence on which this opinion is based is yet to be presented. Kirkpatrick and associates in their papers on particle movement in chicken and human nerve (Kirkpatrick *et al.* 1972; Kirkpatrick and Stern, 1973) imply that the round organelles are something other than mitochondria. In one paper (Kirkpatrick & Stern, 1973), the definite claim is made that 'electron microscopically these are identified as vesicles of smooth endoplasmic reticulum'. Just how this identification was made is, however,

not clear from the paper. The nature of the very large particles, 1·0–1·5 μm in diameter, which are seen moving in cultured amphibian nerves (Berlinrood *et al.* 1972) is also unknown. Published electron micrographs of the cultured tissue showed mitochondria with diameters of about 0·2 μm but no bodies of a larger size. In all, it seems reasonable to conclude that the rod-shaped organelles seen by us and others are mitochondria while the group of round organelles is heterogeneous containing unknown proportions of mitochondria, vacuoles, and other bodies.

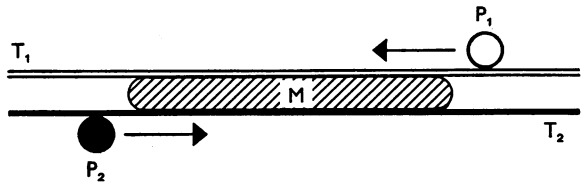
What mechanisms underlie the movement?

Microtubules and neurofilaments have both been implicated in the mechanisms of axoplasmic transport (Schmitt, 1969; Lasek, 1970; Ochs, 1970; Wuerker & Kirkpatrick, 1972). Experiments with colchicine, which binds to microtubule protein (Borisy & Taylor, 1967*a*, *b*), favour the microtubule as having a role to play in fast axoplasmic transport (Dahlström, 1968; Kreutzberg, 1969; Norström, Hansson & Sjöstrand, 1971; Hökfelt & Dahlström, 1971; James, Bray, Morgan & Austen, 1970). In cells other than neurones there is adequate evidence that microtubules are associated with particles that are undergoing saltatory movement (e.g. Freed & Lebowitz, 1970; McGee-Russell & Allen, 1971).

If microtubules are involved in the movement of large intra-axonal organelles then a close association should exist between microtubules and these organelles. Such close associations have been noted between bundles of up to seventeen microtubules and mitochondria (Raine, Ghetti & Shelanski, 1971; Wuerker & Kirkpatrick, 1972; Smith, 1973). It has also been suggested that the relatively small number of organelles seen moving in fibres of the dorsal roots central to the dorsal root ganglion is associated with the paucity of microtubules in these fibres (Smith, 1973). It is possible, then, that the axoplasmic track which is followed by organelles (Text-fig. 10) is composed of bundles of microtubules which are not detected by darkfield microscopy. Such bundles of microtubules would be closely related to the rod-shaped mitochondria alongside which the round organelles often travelled. Immediately the question arises, how may organelles move in opposite directions along the same track? There are two, not mutually exclusive, possibilities. The organelles which travel in opposite directions may have different properties, and parts of the track may be dissimilar.

The possibility that parts of the axoplasmic track, or bundle of microtubules, may differ can be approached by estimating how many microtubules might be required to move organelles of the observed dimensions at the observed velocities. The force, F, required to move a spherical body at a constant velocity in a viscous medium is given by $F = 6\pi\eta rv$,

where η is the coefficient of viscosity of the medium, r is the radius of the body and v is its velocity. Taking the viscosity of cytoplasm as 10 poise, the radius of the particle as 10^{-5} cm, and its velocity as 10^{-4} cm/sec, one obtains a force of 2×10^{-7} dyne. Huxley & Simmons (1971) estimate the force exerted at single cross bridges between the filaments of striated muscle as being at least 2×10^{-7} dyne. Thus, if the mechanisms underlying organelle movement resemble those operating in the contraction of skeletal muscle, it is not unreasonable to think that even a single microtubule might provide the required motive force. The different directions of particle movement could then be explained by postulating two kinds of microtubules within each bundle. These microtubules would have opposite polarities (cf. McIntosh, Helper & VanWie, 1969) and probably different affinities for the outer membrane of different kinds of organelles. Text-fig. 11 summarizes this hypothetical situation. There does seem to be adequate evidence that not all microtubules, even in the same cell, are chemically similar (Margulis, 1973).



Text-fig. 11. A hypothetical arrangement and interaction of intra-axonal structures. Rod-shaped mitochondria (M) are closely associated with microtubules (T_1 and T_2). Microtubules of the types T_1 and T_2 have, or are associated with, force generating mechanisms which act in opposite directions. The microtubules, or their associated force generating mechanisms, have different affinities for different kinds of particles, P_1 and P_2. Particles of each type then move in opposite directions as indicated by the arrows.

Microtubule-particle interactions might underlie our observation that particles tended to hesitate or become arrested at the entry to a node of Ranvier. If microtubules are not very long structures, and if their density in the axoplasm is not proportionately increased at the nodal constriction (cf. Zenker & Hohberg, 1973), then particles may have to compete for carriers across the node. An alternative possibility is that intracellular structures are tightly packed at the node causing a purely mechanical obstruction.

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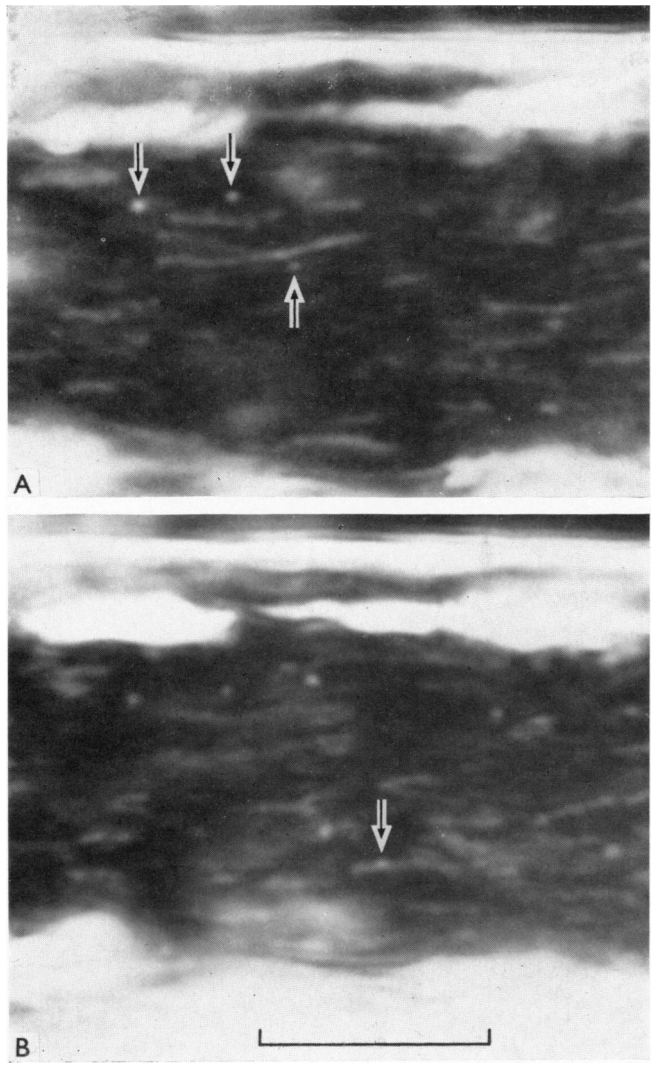
A
B

EXPLANATION OF PLATE

Darkfield photomicrographs of a myelinated axon showing the appearance of organelles within the axoplasm. The two photographs A and B were taken at slightly different positions of focus to demonstrate the large number of organelles which may be detected. In each photograph the bright horizontal bands at top and bottom were caused by light scattered from myelin. In A, round organelles of various diameters may be seen (arrows), as well as axially oriented rod-shaped organelles of various lengths. The very long rod-shaped image between the arrows was caused by the overlapping images of two separate rods. In B the focus was shifted slightly. Images of the two round organelles at the upper left, arrows in A, are still present, but organelles not detected in photograph A may now be seen. A small round organelle is closely associated with one of the rods (arrow). The photographs were taken after organelle motion had been stopped by perfusing the viewing chamber with a buffered 5 % glutaraldehyde solution. Scale bar, 10 μm.