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A STUDY OF THE MOTION OF ORGANELLES WHICH UNDERGO RETROGRADE AND ANTEROGRADE RAPID AXONAL TRANSPORT IN *XENOPUS*

BY Z. J. KOLES, K. D. McLEOD AND R. S. SMITH

From the Division of Biomedical Engineering and Applied Sciences and the Department of Surgery, University of Alberta, Edmonton, Alberta T6G 2G3, Canada

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SUMMARY

1. Axonally transported organelles were detected optically in myelinated axons from *Xenopus laevis* at room temperature (21–23 °C). Details of the motion of organelles which were transported in the retrograde and anterograde directions were studied using filmed records.

2. A group of 133 organelles with a mean retrograde velocity of 0·91 μm/sec was compared with a group of thirty-nine organelles with a mean anterograde velocity of 0·93 μm/sec.

3. Averaged power spectra of the positional deviations about the mean positional change through time were constructed for organelles which travelled in the retrograde and anterograde directions. Most of the power in the two spectra was at frequencies below 0·2 Hz and each contained a single peak at 0·02–0·04 Hz. The power spectrum for retrograde organelle motion had a magnitude about twice that for anterograde organelle motion.

4. Estimates of the instantaneous velocity of organelles which travelled in either direction varied smoothly with time. Instantaneous velocity was not a smooth function of organelle position, (i.e. was 'saltatory').

5. Histograms of the estimates for the groups of organelles whose major motion was retrograde or anterograde were broad, covering a range of about 3 μm/sec, were unimodal, and passed through zero to include a small group of values which indicated motion in the opposite (minor) direction.

6. Organelles spent, on average, more time moving in the minor direction the lower their mean velocity.

7. The variation in instantaneous velocity was greater for organelles which travelled in the retrograde direction than for those which travelled in the anterograde direction. No correlation was found between the variation of instantaneous velocity and the mean velocity of the organelles.

8. Images of organelles occasionally appeared to rotate while the organelle continued to move in the major direction of travel.

9. Evidence is presented that spatially related properties of the axon influence organelle velocity and that this influence is common to organelles which travel in the two major directions.

10. A hypothesis is presented to account for the findings. This supposes that each organelle travels through a stationary axoplasm and is propelled by the resultant of two opposing driving forces whose relative magnitude fluctuates with time. Spatially dependent properties of the axoplasm modify the postulated time-related cycle of motion.

INTRODUCTION

Organelles that are detectable by light microscopy are transported rapidly (approximately 1 μm/sec at room temperature) within axons isolated from the peripheral nerve trunks of both vertebrates and invertebrates (Smith, 1971, 1977; Kirkpatrick, Bray & Palmer, 1972; Cooper & Smith, 1974; Forman, Padjen & Siggins, 1977; Heslop & Howes, 1977). As is the case for the rapid transport of a wide variety of materials which are detected by other means (Grafstein & Forman, 1980), the transport of optically detected organelles is bidirectional. It has been expected that a study of the detailed motion of such organelles would provide some insight into the mechanisms of axonal transport. In fact, the mere verbal description of this motion as being saltatory is used as supportive evidence for a class of ideas in which materials which undergo transport are thought to be intermittently attached to some carrier mechanisms (Ochs, 1975; Goldberg, Goldman & Schwartz, 1976; Goldberg, Schwartz & Sherbany, 1978; Rubinow & Blum, 1980). A comparison of the motion of organelles which move in the anterograde direction with those that move in the retrograde direction might also be expected to be useful in the search for those factors which determine the direction in which organelles move.

The details of organelle movement in axons have been studied quantitatively (Breuer, Christian, Henkart & Nelson, 1975; Forman *et al.* 1977; Leestma & Freeman, 1977), but the results have generated very little insight into the mechanisms which underlie the movement. It is, however, difficult to attribute significance to the quantitative findings since it is possible that some patterns of motion were obscured by the introduction of artifactual high frequency components into the estimates of the velocity of organelles (Koles, McLeod & Smith, 1982). While some general features of the motion of organelles which travel in the anterograde and retrograde directions have been compared (Cooper & Smith, 1974, Hammond & Smith, 1977; Leestma & Freeman, 1977) no detailed comparison has been undertaken; this is in part a consequence of the relative paucity of detectable organelles which travel in the anterograde direction (Cooper & Smith, 1974; Foreman *et al.* 1977).

In the work reported here we have examined the detailed movement of particulate organelles in myelinated axons of the amphibian *Xenopus laevis* using an improved method for estimating the instantaneous velocity of the organelles (Koles *et al.* 1982). The motion of organelles which move predominately in the retrograde direction has been compared with that of organelles which move in the anterograde direction. Organelles which travel in each of the two directions show a basic similarity in the pattern of their motion. This pattern consists of a low frequency variation in the velocity of an organelle about some mean velocity. While the results might be consistent with the proposal that the variable component of velocity is related to either a temporally or a spatially related cycle, it is argued that the observations can

be best explained in terms of a temporal cycle of motion upon which is superimposed a spatial effect that is due to the local mechanical properties of axoplasm. A hypothesis is presented which seeks to explain the observations in terms of the interaction of sites on the surface of a membrane-bounded organelle with polarized force generators which are located in stationary axoplasm.

METHODS

Large diameter (10–22 μm) myelinated axons were isolated at the centre of lengths of sciatic nerve obtained from adult specimens of female *Xenopus laevis*. The arrangement of the isolated-axon preparation in a chamber which allowed intra-axonal organelles to be viewed and filmed at high magnification by dark field microscopy has been described elsewhere (Cooper & Smith, 1974; Hammond & Smith, 1977; Smith, 1980). Preparations were immersed in an oxygenated solution with the composition (mM): NaCl, 112; NaH_2PO_4, 0·45; Na_2HPO_4, 2·6; KCl, 3; $MgCl_2$, 1·6; $CaCl_2$, 2 at pH 7·4 and temperature 21–23 °C. Organelle motion was recorded on black and white 16 mm film (Kodak 4X) at 3 frames/sec with a camera driven by a servo motor.

The procedures for obtaining numerical values for the x (axial to the nerve fibre) and y (transverse) co-ordinates of images of organelles within each motion picture frame and for calculating from these the instantaneous velocities of organelles have been described in detail in another report (Koles *et al.* 1982). These methods may be summarized as follows. The position of organelles was obtained as a pair of x and y co-ordinates at each $\frac{1}{3}$ sec interval. A trend in organelle movement in time was calculated for each of the series of x and y co-ordinates by linear regression. The trends, which yielded measures of the mean velocity of the organelle in each of the two orthogonal directions, were removed from the measurements of x and y to give sets of trend-free displacements. Each set of positional deviations about the mean was treated with a discrete 21-term differentiating filter that attenuated frequencies above 0·3 Hz. Instantaneous velocities for the organelles were obtained by adding the result of the band-limited differentiation to the appropriate estimates of mean velocity.

The main additional method was the construction of a contour map of the distribution, within the focal plane of the microscope, of the instantaneous velocities of organelles which moved in the retrograde direction in one axon (Axon I, Table 1). The mapping procedure, a computer program called Surface II (Sampson, 1975), was implemented on the University of Alberta's Amdahl 470 computer. An optical section of the axon measuring about 18×38 μm contained 4375 irregularly spaced estimates of instantaneous velocities derived from forty-two organelles whose trajectories had been recorded during one 30 min period. The area to be mapped was divided into 684 cells of 1 μm side; each corner of each cell is referred to as a node of the grid. A value for velocity was assigned at each node by taking a weighted average of the individual velocity estimates which were within some specified distance of the node. Individual velocity estimates were taken in sequential order of their distance (D) from the node out to a maximum distance of 1·5 μm. At least one estimate had to lie within 1·0 μm of the node with a minimum of three and a maximum of twelve velocity estimates being used for the value assigned to the node. The weighting factor applied to each individual estimate of instantaneous velocity was $1/D$. The precise values for this research and averaging procedure were selected to avoid artifactually produced axially arranged contours and also to avoid low average values of velocity being linked to a low density of data points.

RESULTS

General characteristics of organelle motion

The organelles whose motion is treated in this study were particulate; their images were generally circular or elliptical with a major axis which measured from 0·2 to 1 μm. Rod-shaped organelles with a major axis of up to 8 μm were occasionally seen to move either intermittently or continuously across the field of view (e.g. Cooper & Smith, 1974). Most of the particulate organelles moved throughout their measured

trajectories in a direction that was approximately parallel to the long (x) axis of the nerve fibre or in a course which undulated smoothly about such an axis. Large amplitude, sudden movements in the transverse (y) direction were rare; such transverse movements had been described elsewhere (Smith & McLeod, 1979) and are not included in the analyses which follow. All particulate organelles, whether they move predominately in the retrograde or anterograde directions, moved in an

TABLE 1. Means, standard deviations (S.D.) and ranges of the average velocities in the x direction of organelles that travelled in the retrograde and anterograde directions in each of ten axons, A–J. Number of organelles, n.

	Retrograde Velocities					Anterograde Velocities				
				Range					Range	
Axon	n	Mean (μm/sec)	S.D.	min	max	n	Mean − 1 (μm/sec)	S.D.	min	max
A	10	0·49	0·38	0·06	1·37	2	0·98	0·33	0·75	1·21
B	10	0·93	0·41	0·28	1·42	4	1·10	0·62	0·58	1·89
C	11	0·83	0·41	0·30	1·44	2	0·67	0·06	0·63	0·71
D	10	1·07	0·40	0·57	1·91	3	0·56	0·14	0·41	0·68
E	10	0·86	0·34	0·35	1·43	7	0·67	0·06	0·59	0·75
F	10	1·12	0·35	0·71	1·66	1	1·14	—	—	—
G	10	1·16	0·43	0·39	1·68	3	0·89	0·42	0·56	1·36
H	10	0·91	0·32	0·45	1·41	3	1·01	0·60	0·63	1·71
I	42	0·81	0·32	0·23	1·59	9	1·04	0·60	0·27	1·93
J	10	0·98	0·42	0·32	1·81	5	1·07	0·72	0·25	1·95

TABLE 2. Means and standard deviations (S.D.) of velocities in the x (V_x) and y (V_y) directions for 133 organelles which travelled in the retrograde direction and thirty-nine organelles which travelled in the anterograde direction.

	Retrograde		Anterograde	
Component	Mean (μm/sec)	S.D.	Mean (μm/sec)	S.D.
V_x	0·91	0·38	−0·93	0·47
V_y	0·00	0·11	0·02	0·10
V_s	0·91	0·38	−0·93	0·47
	$n = 133$		$n = 39$	

V_s, the vector sum of V_x and V_y.

irregular 'saltatory' manner and commonly showed brief reversals in their direction of travel. In accordance with the terminology introduced earlier (Forman *et al.* 1977), the preferred direction of travel for any organelle will be called the *major* direction of movement while the reverse will be called the *minor* direction. Retrograde motion has been arbitrarily assigned a positive sign. The slopes of the regression lines fitted to plots of organelle position with respect to time defined the average velocities of the organelles in the x and y directions.

Table 1 presents the means of the average x velocities for organelles travelling in the retrograde and anterograde directions in each of ten axons. Over-all values for

the velocity components of 133 organelles whose major direction of travel was retrograde and thirty-nine organelles whose major direction of travel was orthograde are given in Table 2. An F test showed that the variances of the pooled average x velocities of organelles which moved in the retrograde and anterograde directions were not equal ($P < 0{\cdot}05$); the mean velocities of the two groups were, however, not significantly different.

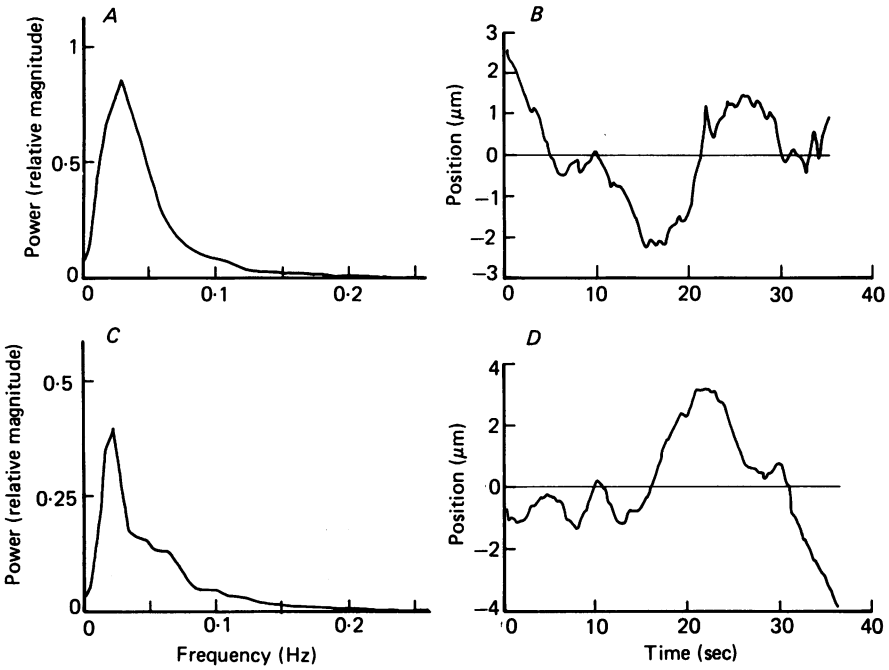

Fig. 1. Properties of the details of the motion of organelles in the axial (x) direction of movement. The average motion of each organelle (the trend, or fitted regression line to the position–time data) was removed from the data and the residual motion was analysed in terms of its frequency power spectrum. Average power spectra of the trend-free motion of 133 organelles whose major direction of movement was retrograde (A) and thirty-nine organelles whose major movement was anterograde (C) demonstrate the low frequency character of the detailed motion. Examples of the trend-free motion of individual organelles are shown: B, an organelle whose major motion was retrograde, and D, an organelle whose major motion was anterograde. The horizontal line at zero position in B and D represents the trend-free average position of the organelle.

Positional deviations about the trend

Removal of the trend (the fitted regression line) from position–time plots of organelle motion allowed some characteristics of the details of motion to be examined in isolation from the average properties of organelle movement. Examples of the trend-free variations in position with time are shown in Fig. 1 B and D for the x motion of two organelles, one moving in the retrograde direction and one moving in the anterograde direction. The power spectra of the positional deviations about the trend were computed for the 133 retrogradely moving organelles and an average power

spectrum was formed from each group (Fig. 1 *A* and *C*). Both averaged power spectra show a single main peak at a frequency of 0·02–0·04 Hz. If this may be taken as the average saltatory frequency, then, taking the average organelle velocity as 0·92 μm/sec (Table 2), the average length of a single saltation was in the range 20–45 μm for organelles which travelled in either the orthograde or retrograde directions. An examination of individual power spectra produced no evidence that

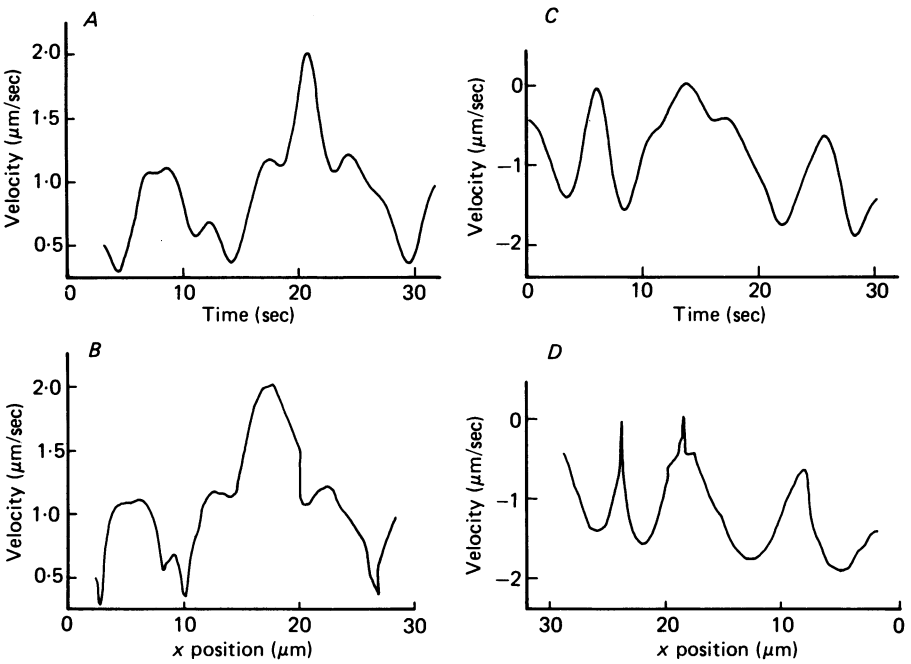

Fig. 2. Instantaneous velocities of individual organelles with major movement in the retrograde (*A*, *B*) and anterograde (*C*, *D*) directions. Velocities are plotted for each organelle with respect to time(*A*, *C*) and with respect to the *x* position of the organelle (*B*, *D*). In this and subsequent Figures retrograde motion is assigned a positive sign.

the positions of the main spectral peaks were related to the average velocity of the organelles. Only one difference was noted in the pattern of movement of particles which travelled in opposite directions: the low frequency power in the trend-free deviations in the *x* positions of organelles which moved in the retrograde direction was, on the average, about twice that for organelles which moved in the anterograde direction (Fig. 2*B* and *D*).

Instantaneous velocities

The instantaneous velocities of organelles which travelled in the retrograde or anterograde directions had very similar features. Organelles moving in either direction showed a smooth variation of velocity with respect to time (Fig. 2*A* and *C*) while velocity plotted as a function of the position of the organelle (Fig. 2*B* and *D*) varied in a more abrupt manner. It is likely that the abrupt changes of velocity

with respect to position give rise to the verbal descriptions (e.g. Cooper & Smith, 1974; Forman *et al.* 1977) of organelle motion as being saltatory. The velocity-time curves occasionally crossed the zero-velocity value to result in a change in the sign of velocity and a movement of the organelle in the minor direction of travel (e.g. Fig. 6*D* of this paper and Fig. 4*A* and *C* in Koles *et al.* 1982). The tendency of organelles to travel

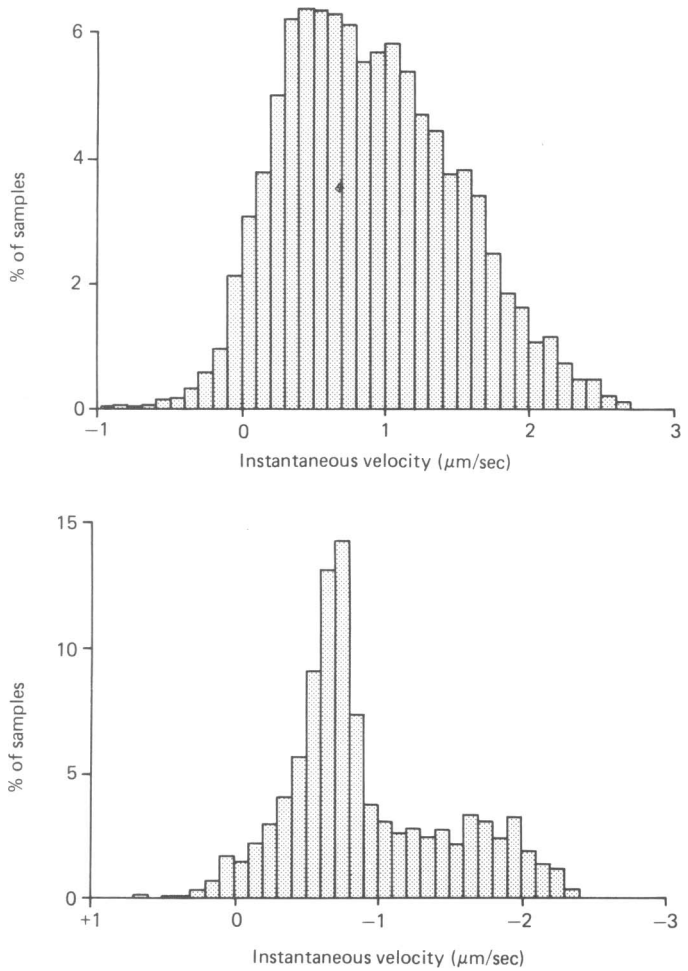

Fig. 3. Histograms of the distribution of instantaneous velocities of 133 organelles whose major motion was retrograde (above) and thirty-nine organelles whose major motion was anterograde (below).

in the minor direction is illustrated in the histograms of Fig. 3 which show the distributions of instantaneous velocities for the group of 133 organelles which travelled in the retrograde direction and for the group of thirty-nine organelles which travelled in the anterograde direction. Instantaneous velocities due to motion in the minor direction of travel accounted on average for 4·5% of the distribution of

instantaneous velocities for organelles whose major directionality was retrograde and 3·1 % for organelles whose major direction was anterograde. Distributions of the instantaneous velocities of individual organelles were similar to those shown in Fig. 3; the distributions were broad and unimodal. The percentage of time that individual organelles spent moving in the minor direction was correlated with the mean velocity of the organelle so that slower organelles tended to spend more time in reversed motion (Fig. 4).

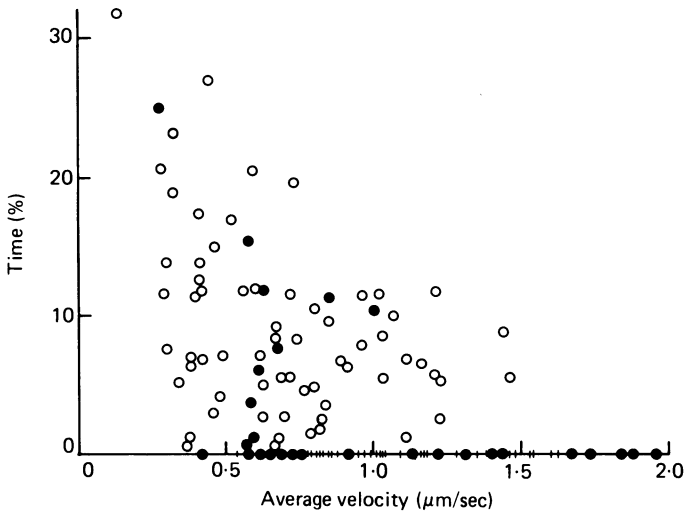

Fig. 4. The percentage of time that each organelle spent moving in the minor direction plotted as a function of the average velocity of the organelle. Open circles and short vertical lines on the abscissa represent values for organelles whose major movement was retrograde. Filled circles are values for organelles whose major movement was anterograde.

An analysis of the distribution of instantaneous velocities for each group of organelles showed that the standard deviation of the instantaneous velocities of organelles travelling in the retrograde direction was $0{\cdot}45 \pm 0{\cdot}16$ μm/sec (mean of standard deviations $\pm$ S.D. of standard deviations, $n = 133$) while that for organelles travelling in the anterograde direction was $0{\cdot}30 \pm 0{\cdot}19$ μm/sec ($n = 39$). Thus, organelles which travelled retrogradely showed significantly more variation ($P < 0{\cdot}01$) in their instantaneous velocities than did those which travelled anterogradely. This result is in line with the finding that the power in the spectrum of the positional deviations about the trend was greater for organelles which travelled in the retrograde direction (Fig. 2).

For individual organelles there was no significant correlation between the magnitude of the standard deviations of instantaneous velocity and the average velocity of the organelle. Hence, it appears that the velocity of any organelle, whether it moves in the retrograde or anterograde direction, may be viewed as being composed of a constant term which may differ for different organelles, plus an independent term that varies with time.

Evidence for lack of organelle polarity

While any given organelle travelled in a preferred direction, certain observations suggested that this was not due to a polarity of the organelle. On four occasions organelles which gave rise to an elliptical image profile were seen to turn end over end while continuing to move in the major retrograde direction (Fig. 5*A*). In each of the four cases the organelle turned over once; the tumbling was not continuous.

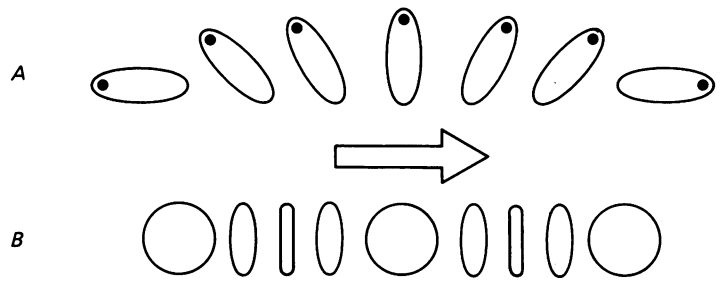

Fig. 5. Diagrams to show changes in the image of organelles which suggest that organelles may possess no directional polarity. Arrow indicates progression of change in position and time. *A*, elliptical images were sometimes seen to turn end over end. Dot indicates the end of the organelle which was initially trailing and became leading. *B*, disk to line changes in the image which suggested that the organelle was shaped as a plate and spun on its *y* axis.

There was no indication that the phenomenon could have been due to a change in the shape of the organelle. On a number of other occasions large ($\sim$ 0·5–1·0 μm diameter) circular images assumed the sequence of shape changes shown in Fig. 5*B*. In some of these instances the change in the image was repeated several times across the field of view. While the changes in image shape shown in Fig. 5*B* might have been caused by modelling of a flexible organelle, a lack of appropriate dimensional changes suggested that it was more likely that the image represented a disk-shaped organelle which turned about its *y* axis.

Spatially related aspects of organelle motion

Whether the motion of organelles is influenced by spatially related properties of the axon was investigated initially by studying pairs of organelles which followed very similar pathways within the same axon. Examples of such similar pathways are shown in Fig. 6*A* and *C*; the corresponding plots of organelle velocity with respect to the *x* position within the axon are shown in Fig. 6*B* and *D*. Some of the variations in velocity in the pairs of organelles did appear to be related to the position of the organelles within the axon. The presumed spatial dependence of organelle velocity was further investigated by constructing a contour map in which the instantaneous velocities of forty-two organelles which travelled in the retrograde direction (Axon I, Table 1) were related to the position of the organelles within the axon. Our assumption was that if no strong spatial dependence of organelle velocity was present then the averaging involved in constructing the contour plot would produce a map

with few, widely spaced, contours. This was not the case (Fig. 7); the contours spanned the range of organelle velocities and thus demonstrated regions within the axon in which retrogradely moving organelles tended to move rapidly or more slowly than the average. While the contours of the intermediate velocity values tended to be longitudinally oriented, the extremes of velocity occurred in discrete rounded areas. The regions of preferred high or low velocity did not bear any noticeable

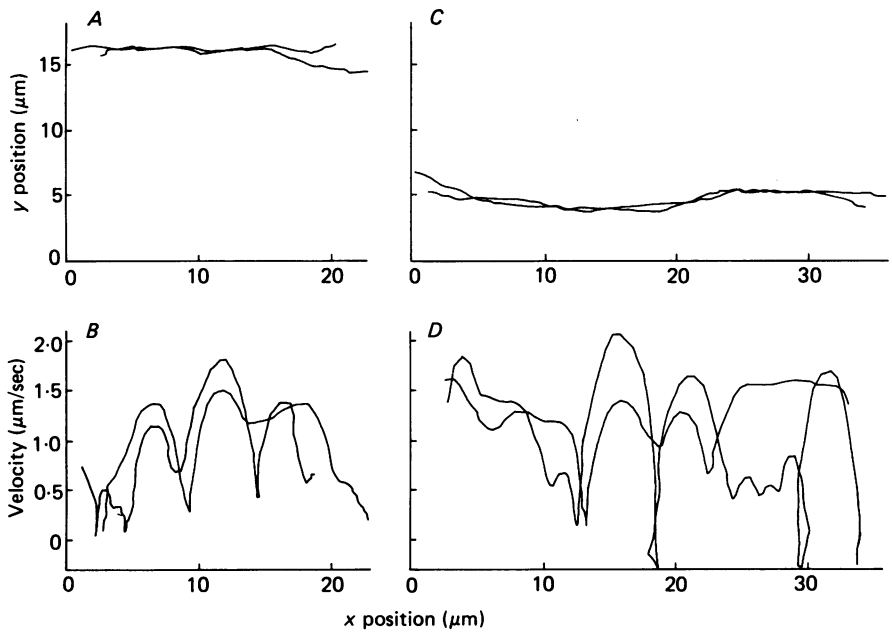

Fig. 6. The motion of two pairs of organelles within the same axon. Movement of the organelles was retrograde, left to right. In *A* and *C* the trajectories of the two pairs of organelles are plotted to show the extent to which the trajectories overlapped within the projection of the axon viewed by the microscope. The corresponding plots of organelle velocity with respect to the *x* position of the organelles, *B* and *D*, demonstrate a general similarity in the changes in velocity for each pair of organelles. Note that one of the organelles in *D* underwent brief changes in the direction of movement at approximately the 18 and 29 μm positions.

relationship to any structural features of the axon that could be detected in the light microscope such as the distance from the wall of the axon or the position of stationary rod shaped organelles (mitochondria).

While the nine organelles which were tracked in the anterograde direction in the same axon did not produce enough data points for the construction of a contour plot, some of the individual curves of velocity with respect to position showed similar changes in velocity at similar positions in the axon. The variations in velocity with position could also be related to the contour plot of Fig. 7; this suggests that spatially related properties of the axoplasm have a common influence on the velocity of organelles which move in either the retrograde or anterograde directions.

DISCUSSION

The measurements and observations which are presented in this paper contain no unambiguous indications of the mechanisms which may underlie organelle motion in axons, but they nevertheless constitute a description which allows an apparently complex pattern of movement to be understood in fairly simple terms. The discussion which follows will briefly outline the relationship of our findings to those of other workers. Then, our work will be treated in terms of one of the assumptions that is currently applied to rapid axonal transport, namely, that transport reflects the movement of membrane-bounded organelles through an essentially stationary axoplasm.

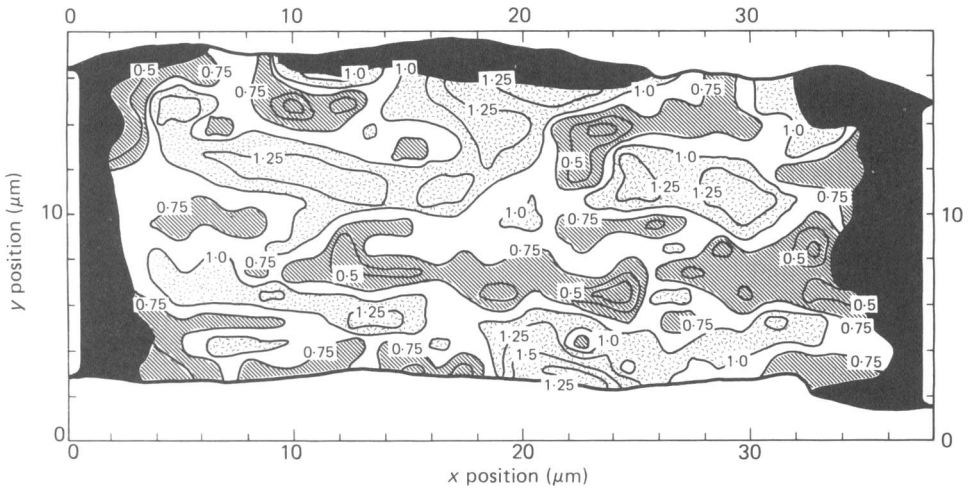

Fig. 7. Contour map of organelle velocity with respect to organelle position in the microscope field of view. The map is constructed from 4375 estimates of instantaneous velocity which were obtained from forty-two organelles whose movement was retrograde (Axon I, Table 1). Outline of axon shown as a heavy line. Areas of the axon for which no velocity estimates were obtained shown in black. The velocity contours are drawn at intervals of 0·25 μm/sec. Most of the contour lines are labelled, in units of μm/sec. For convenience in viewing the map, the regions in which organelle velocity was predominately low (0·75 μm/sec or less) are shaded dark while the regions in which organelle velocity was high (1·0 μm/sec or more) are shaded lightly. The velocity interval 0·75–1·0 μm/sec is white.

Earlier descriptions of organelle movement in axons have emphasized the saltatory character of the motion (Berlinrood, McGee-Russel & Allen, 1972; Kirkpatrick *et al.* 1972; Cooper & Smith, 1974; Breuer *et al.* 1975; Heslop & Howes, 1977; Forman *et al.* 1977; Leestma & Freeman, 1977). The work presented in this report confirms this description with the additional observation that the saltatory character of the motion appears to be most strongly expressed when the instantaneous velocity of an organelle is viewed as a function of organelle position. On the other hand, organelle velocity is a smoothly varying function of time with an average fundamental frequency over all the organelles studied of between 0·02 and 0·04 Hz. This low

frequency variation in velocity may have been one of the factors which prevented the detection of patterned motion by other workers: while portions of a saltatory cycle might often be observed within a high power microscopic field of view, the fundamental frequency of saltation gives rise to a spatial period that is close to or even greater than the width of the field.

The second striking characteristic of organelle motion in axons is the tendency of the organelles to undergo brief reversals in direction. This tendency has been analysed quantitatively by Breuer *et al.* (1975) and Forman *et al.* (1977). An important finding was that of Forman and co-workers who noted that the distribution of the instantaneous velocities of organelles which travelled in the retrograde direction was unimodal, and that those velocities which represented movement in the minor direction occupied one tail of a broad distribution. This result could have been due in part to the use of a method for obtaining instantaneous velocity that is demonstrably prone to error (Koles *et al.* 1982). The finding of Forman and co-workers is, however, confirmed by the present results and is now extended to include the behaviour of optically detectable organelles which move in the anterograde direction. A broad unimodal distribution of instantaneous velocities is not consistent with the proposal (Goldberg *et al.* 1976, 1978; Rubinow & Blum, 1980) that axonal transport in general, and the saltatory motion of large organelles in particular, is caused by the intermittent attachment of the organelles to a carrier which moves at a constant velocity. Clearly, that hypothesis predicts two modes of instantaneous velocity, one at zero velocity and one at the velocity of the carrier–organelle complex.

Further discussion of the results contained in this paper requires some consideration of the general nature of the mechanism by which organelles are transported in axons. There are two such general mechanisms: one assumes that organelles are carried passively in a moving stream of liquid cytoplasm (e.g. Gross, 1975), and the other supposes that an organelle moves through an essentially stationary ground substance by the action of some force which is developed between the ground substance of the cell and the external surface of the organelle (Schmitt, 1968; Cooper & Smith, 1974). No experimental test has yet been conducted which would unambiguously distinguish between the two possibilities; however, the behaviour of organelles under certain conditions, as at the site of a constriction or a crush lesion in an axon (Smith, 1980), is more in line with the idea that large axonal organelles move through a stationary matrix and that cytoplasmic streaming is not involved. At this time the idea that organelles move through a stationary matrix is also more tractable to analysis. If one assumes that the dynamic viscosity of the medium surrounding the organelles is of the order of 1 Nsec/m^2, then the Reynolds number of an organelle with the observed dimensions and which moves at a velocity of 1 μm/sec is of the order of 10^{-9}. The very small value of the Reynolds number implies that such organelles are essentially inertialess and that this statement does not depend closely on the precise values of the parameters which are used in estimating the number. Such an organelle, if acted upon by an external force (F) will instantly attain a velocity (V) such that the retarding force due to viscous drag (KV, where K is a constant which depends on the viscous properties of the axoplasm and the geometry of the organelle) is in balance with the driving force: $F = KV$. The velocity of the organelle is then a measure of the combined effect of the retarding and driving forces which

act on the organelle, and a variation in the velocity of the organelle will be produced if either or both of these forces vary.

Our measurements of organelle velocity in axons suggest that the observed saltatory character of the motion can be explained either as a smooth variation of velocity with time or as an irregular, but still periodic, variation of velocity with respect to the position of the organelle. The evidence (Fig. 7; and Leestma & Freeman, 1977) indicates that the velocity of an organelle is dependent on the properties of the axoplasm through which it travels. However, neither our current knowledge of the structure of axons nor the distribution of velocity contours in Fig. 7 provide support for the idea that spatially related effects could explain a low frequency, periodic oscillation in organelle velocity. Neither does the assumption that spatially related variations in the mechanical properties of axoplasm are entirely the cause of saltatory motion contain any explanation for the observed tendency of organelles to reverse their direction of travel during those times at which organelle velocity is low. It is possible, however, to explain the observed characteristics of motion if it is assumed that organelles are subject to a driving force which varies smoothly with time and that this results in their motion through a stationary medium whose local properties also affect the velocity of the organelles.

Fig. 8 is a diagrammatic illustration of the proposal that the motion of an organelle may be described in terms of a periodic variation in velocity which is superimposed on some steady velocity. For convenience it is assumed that the retarding force is constant so that the left-hand ordinate of Fig. 8 can be scaled in units of velocity or driving force. As suggested by our results, the variable component of velocity has a magnitude which is not related to the magnitude of the mean velocity. In addition, it is clear that an organelle with the illustrated kind of behaviour would spend more time moving in the minor direction the closer its mean velocity was to zero. The constant period and amplitude of the variable component of velocity shown in Fig. 8 are intended to represent average properties of the variable component. To describe organelle motion more realistically the statistical properties of the velocity estimates would have to be taken into account (Figs. 2–4). Nevertheless, Fig. 8 is, within these limitations, completely consistent with our results.

While the proposal given above and diagrammed in Fig. 8 appears to constitute an adequate descriptive explanation of the characteristics of organelle motion in axons, some extension of the proposal is required if these characteristics are to be attributed to the function of particular components of axoplasm. The evidence which has been presented indicates that organelles may rotate about an axis while proceeding in the major direction of travel, and this suggests that organelles themselves have no directional polarity. It is assumed, then, that the directionally polarized elements of force generation reside in the axoplasm (possibly in association with microtubules: Cooper & Smith, 1974; Hammond & Smith, 1977) and that the direction in which a given organelle will travel is specified by some property of the surface of the organelle such that it interacts with the appropriate force generator. If the organelle is covered with a mosaic of sites that are of two kinds, one kind (a retrograde site) capable of interacting with, and possibly activating, a 'retrograde' force generator, and a second kind (an anterograde site) which interacts with an 'anterograde' force generator, then the magnitude of the force which moves the

organelle and its direction of action will depend on the number of active sites of each kind. Suppose that a total of n sites of either kind is available for interaction with force generators that produce some unit force f; some proportion (P_r) of the n sites is of the retrograde kind and the proportion contributed by the rest ($P_a = 1 - P_r$) is of the anterograde kind. Then the magnitude and direction of the net force (F) is given by $F = (P_r - P_a)nf$. Thus, the velocity of the organelle would be maximal in

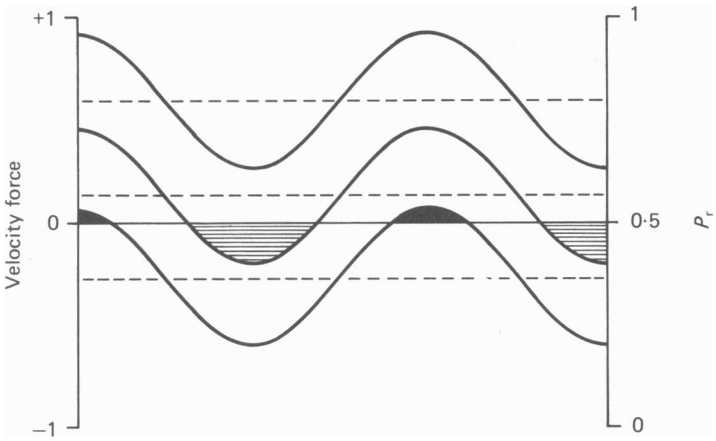

Fig. 8. Diagram which summarizes the experimental results and which presents a hypothetical explanation of the results. Left hand ordinate scaled in arbitrary units of velocity or force; positive sign indicates motion or action in the retrograde direction, negative sign anterograde. Right hand ordinate scaled in P_r, the proportion of active sites on the surface of the organelle which produce a force acting in the retrograde direction. The abscissa is time. The results are summarized by considering a simplified expression of the motion of three organelles (heavy lines). Each organelle, whether its major direction of motion is retrograde or anterograde, shows a low frequency variation in velocity. The amplitude of the low frequency variation in velocity of each organelle is not related to its mean velocity (mean velocity is indicated by dashed horizontal lines). The probability that an organelle moves in the minor direction is related to the mean velocity of the organelle. Minor motion by an organelle whose major movement is retrograde is indicated by horizontal shading. Minor motion by an organelle whose major movement is anterograde is shown in black.

either direction when either P_r or P_a were equal to unity, and the organelle would remain stationary in the case where $P_r = P_a = 0{\cdot}5$. This hypothesis is illustrated in Fig. 8 by the inclusion of an ordinate, scaled in P_r, at the right hand side of the Figure. It is assumed that the variable component of velocity is caused by a variation of P_r and P_a with time.

A simple prediction follows from this hypothesis. If the major direction of motion of an organelle were to change from, say, retrograde to anterograde then this might be accomplished by an alteration in the mean level of P_r from values greater than 0·5 to values less than 0·5. If the periodic fluctuation in P_r remains roughly constant during this change then the organelle should pass through a condition in which it oscillates in the longitudinal direction about some fixed position. Some evidence has been presented (Smith, 1980) that organelles may change their major direction of

movement when they reach a crush lesion in the axon, a phenomenon which has also been reported for other axonally transported materials (Bray, Kon & Breckenridge, 1971; Lubinska & Niemierko, 1971; Abe, Haga & Kurokawa, 1974; Bisby & Bulger, 1977). In the case of axonal organelles it was noted (Smith, 1980) that the change in direction appeared to be preceded by a period during which the organelles showed longitudinal oscillatory movement with an amplitude of roughly 10 μm.

The hypothesis which has been presented above might be tested by attempting to block selectively the interaction of one of the postulated kinds of sites on the organelle with its matching force generator. The expected outcome is quite clear: the organelle would either continue to move in the same direction with an increased velocity and a complete elimination of movement in the minor direction, or it would reverse direction. A modification of the axoplasmic concentration of calcium, to which anterograde transport is sensitive (Ochs, Worth & Chan, 1977), and of magnesium to which the retrograde transport of organelles appears to be sensitive (Christian, 1975; Smith & Snyder, 1980) may allow such a test to be carried out.

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