VELOCITY DISTRIBUTIONS OF THE STREAMING PROTOPLASM IN *NITELLA FLEXILIS*

R. V. MUSTACICH *and* B. R. WARE, *Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138*

ABSTRACT Laser light is Doppler-shifted in frequency by the streaming endoplasm of living cells of *Nitella flexilis*. The frequency spectrum of the scattered light can be interpreted as the histogram of velocities within the organism, with the exception of the intense low-frequency portion of the spectrum. We demonstrate that the lowest-frequency component is the result of amplitude modulation of the scattered light by the array of chloroplasts in the cell. Measurement of the streaming endoplasm in a photobleached "window" region allows correction of the frequency distribution for the modulation component. The complete velocity histogram for the streaming endoplasm is calculated directly from the corrected frequency distribution. Measurements of vacuolar and endoplasmic motions show that the tonoplast, the membrane separating the vacuole and the endoplasm, seems to be flowing along with the endoplasm and vacuolar sap. Placing the cell in medium containing ATP in concentrations greater than 10^{-3}M greatly increases the contribution of low velocities to the velocity histogram. Cytochalasin B at high dosages (10–50 μg/ml) does not noticably change the shape of the velocity histogram, while at low dosages (1 μg/ml) there is an increase in the contribution of low velocities to the velocity histogram. Colchicine in high concentrations (1%) has no observable effect on the velocity histogram.

INTRODUCTION

In previous publications we have demonstrated the utility of laser Doppler spectroscopy for measurement of protoplasmic streaming velocities and velocity distributions in *Nitella* (18, 20). Laser light scattered from the streaming protoplasm is shifted in frequency by the Doppler effect. The magnitude of the frequency shift is proportional to the velocity of the scattering particle as viewed in the scattering plane. The distribution of frequencies, the frequency spectrum, is rapidly detectable with great accuracy, providing a determination of the distribution of velocities of scatterers. The frequency spectrum of the scattered light from *Nitella* includes a peak centered at a Doppler shift frequency corresponding to the normally cited streaming velocity. Fig. 1 shows a frequency spectrum collected from an internodal cell of *Nitella*. The Doppler-shifted peak frequency corresponds to an observed streaming velocity of 72 μm/s. In addition to the peak in the spectrum, there is some intensity at intermediate frequencies and considerable intensity at frequencies below 10 Hz. The low-frequency component of the spectrum is present in all spectra recorded from *Nitella*. Possible explanations sug-

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gested for this low-frequency intensity have included contributions from slow vacuolar motions, low velocities at the ectoplasm-endoplasm interface, the wavelike motion of undulating filaments, modulation of the light intensity by interior cell structures, and vibrations of parts of the cell. We present evidence that this low-frequency component in the laser Doppler spectrum from *Nitella* is the result of modulation of the scattered-light intensity by the chloroplast array of the internodal cell. Removal of the chloroplasts results in dramatic reduction of the low-frequency intensity. A calculation of the expected modulation spectrum based upon the dimensions of the chloroplast array is shown to be consistent with observed spectra. Measurements of vacuolar motions by light microscopy are also discussed to assess vacuolar contributions to the low and intermediate frequencies observed in the laser Doppler spectrum from *Nitella*.

Several studies have been devoted to the effects of ATP, colchicine, and cytochalasin B on streaming in *Nitella* and *Chara* (4, 5, 13, 26, 32, 34). The streaming in *Nitella flexilis* has been shown to be stimulated slightly by a narrow range of concentrations of ATP (26). Higher ATP concentrations inhibit streaming and lower concentrations have no effect. Cytochalasin B is a potent inhibitor of protoplasmic streaming in *Nitella* and *Chara*, and is thought to interact with endoplasmic myosin to halt streaming (24, 29). Colchicine, an agent capable of disrupting microtubules, has been shown to have no effect on protoplasmic streaming in *Nitella* and *Chara* (4, 13). We have therefore measured the changes in the Doppler spectrum induced by these drugs to determine the sequences of changes in the velocity distribution.

METHODS

Laser light scattered from particles in the streaming protoplasm of living cells is frequency-analyzed to obtain the velocity distribution. The frequency shift in Hz for each particle is equal to $|\mathbf{K} \cdot \mathbf{v}|/2\pi$ where $\mathbf{v}$ is the streaming velocity of the particle and $\mathbf{K}$, the scattering vector, is equal in magnitude to $(4\pi n/\lambda) \sin \theta/2$, where n is the refractive index of the scattering medium, λ is the wavelength of the incident light *in vacuo*, and θ is the scattering angle (for detailed discussion of scattering geometry and data interpretation see 20). When the streaming direction is oriented perpendicular to the incident light and in the scattering plane, the spectrum of the scattered light from *Nitella*, measured at an angle θ, may be regarded as a velocity histogram for frequencies above 10 Hz with the $|\mathbf{v}|$ given by $\lambda S/(n \sin \theta)$ where S is the Doppler shift in hertz. In previous publications (18, 20) we demonstrated the validity of the laser Doppler method for measuring the histogram of velocities of flowing particles in living cells and in model systems whose flow profile is known. We have also shown that the spectrum from *Nitella* is not substantially broadened by diffusion and can therefore be interpreted as the velocity histogram within the organism.

Samples of *Nitella flexilis* were obtained from Carolina Biological Supply Co. (Burlington, N.C.) and grown in artificial pond water in normal fluorescent room light. For the light-scattering experiments, the internodal cells were excised and bound gently in special holders so that the direction of streaming was perpendicular to the incident light. Each cell was surrounded by artificial pond water throughout the experiment. Incident laser light of wavelength 632.8 nm was attenuated to a power less than 0.02 mW and focused to a diameter of approximately 100 μm, thereby illuminating a very small portion of a single cell. The resulting intensity was about 250 mW/cm^2, well below photoresponse thresholds (20). The scattered light

was detected by a photomultiplier tube by heterodyne detection, in which scattered light from stationary parts of the cell (the cell wall and chloroplast array) is "mixed" with the Doppler-shifted scattered light from moving scatterers. Low-frequency difference beats equal to the Doppler shifts are produced by the photomultiplier tube. The photocurrent was frequency analyzed with real-time efficiency by a Saicor 51-B time-compression spectrum analyzer (Honeywell, Inc., Denver, Colo.). Experimental details of the apparatus are described elsewhere (20). A digital circuit designed to record the instantaneous spectral position of a Doppler-shifted peak was used to monitor the changing velocities of protoplasmic streaming induced by the addition of drugs. Details of the circuit have been given elsewhere (19).

RESULTS

A heterodyne spectrum of the light scattered from an internodal cell of *Nitella flexilis* is shown in Fig. 1. An incident beam of 100 μm diameter was directed through the center of the cell midway between the so-called "indifferent zones," the boundaries between the two opposite streaming directions. The scattering angle was 28.8° and the temperature was 25°C. The peak-frequency Doppler shift is 93 Hz, which corresponds to a velocity of 72 μm/s. Very little relative intensity is observed at frequencies much greater than the Doppler-shifted peak frequencies. Intermediate streaming velocities are evidenced from the frequency spectrum by the intensity in the range from 20 to 60 Hz, while the signal below 20 Hz has a high intensity relative to the rest of the spectrum.

Spectral analysis of the scattered light observed at zero scattering angle should yield no Doppler shifts since $|\mathbf{K}| = 0$ when $\theta = 0°$. Hence, the spectrum in Fig. 1 should vanish if it is the result of Doppler shifts from moving particles. The spectrum observed at zero angle from a normally streaming internodal cell of *Nitella* is shown in Fig. 2*a*. The entire spectrum vanishes except for the low-frequency intensity, which must not be a result of Doppler shifts. The half-width at half-height of the spectrum in

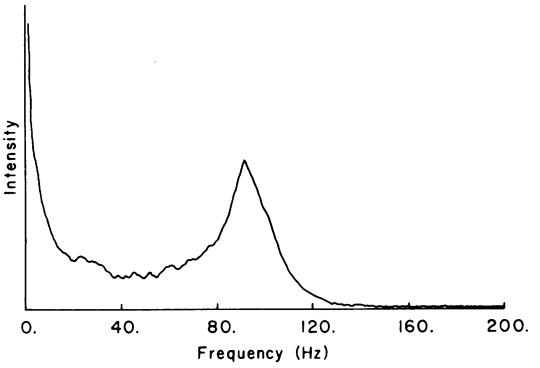

FIGURE 1 Frequency spectrum of the scattered light from an internodal cell of *Nitella flexilis*. The scattering angle was 28.8° and the temperature was 25°C. The peak frequency at 93 Hz corresponds to a streaming velocity of 72 μm/s. There is a narrow distribution of frequencies about the peak frequency (93 Hz), with considerable intensity at lower frequencies. Little intensity is observed at much higher frequencies.

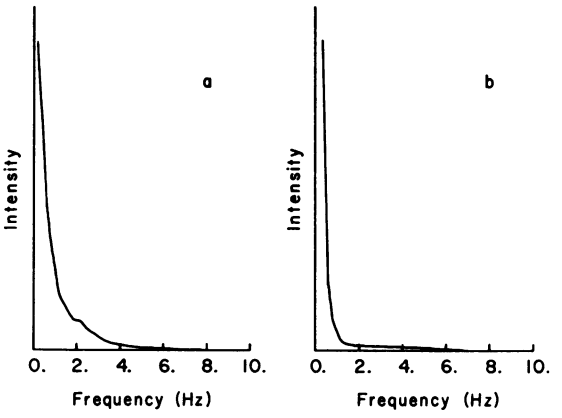

FIGURE 2 Spectrum of scattered light from *Nitella flexilis* at zero scattering angle. Spectrum *a* shows the experimentally observed spectrum at $\theta = 0°$ scattering angle. Chloroplasts partially block the view of the scattering particles, which results in a "blinking" of the scattered light as streaming particles pass behind the chloroplast array. This results in an amplitude modulation of the scattered light. Spectrum *b* is a theoretical amplitude modulation spectrum calculated from the dimensions of the chloroplast array and the velocity distribution of the endoplasm.

Fig. 2*a* is approximately 0.5 Hz. This spectrum vanishes if the streaming inside the cell is halted, so it must be related to particle motion, though not through the Doppler effect. We interpret this spectrum to be the result of streaming scatterers, passing behind the array of stationary chloroplasts, which result in a modulation of the scattered light intensity. When particles are blocked from the detector's view by the chloroplasts, their scattering intensity is diminished. The cell wall is relatively transparent between chloroplasts. This creates a "blinking" effect that introduces low-frequency amplitude modulations to the photocurrent spectrum.

To determine if the chloroplast array is a reasonable source of the observed modulations, we photographed an internodal cell of *Nitella flexilis* to determine an outline of the chloroplast array. A 28 by 160 μm area of the array was digitized into 9,000 points to create a matrix representation of the chloroplasts. This matrix representation was Fourier-transformed by computer to give the power spectrum of the spatial frequencies in cycles per micrometer of the array in the streaming direction. Scaling the abscissa by a velocity (micrometers per second) gives directly the modulation spectrum in hertz for scattering particles of the selected velocity. The spatial frequency spectrum was integrated over the velocity distribution represented by Fig. 1 to yield the spectrum shown in Fig. 2*b*. This integration assumed a uniform spatial distribution of streaming velocities viewed through the array of chloroplasts. The calculated spectrum is of the same form as the spectrum observed experimentally at zero angle. Assumptions such as the opacity of the chloroplasts relative to the cell wall and endoplasm and the uniform spatial distribution of velocities behind the chloroplast array, combined with round-off error in digitizing the chloroplast array and experimental errors

in measurement of this very low-frequency component, may account for the difference in width between the observed and calculated low-frequency spectral component at zero scattering angle.

The width of the low-frequency component of the spectrum increased with increasing scattering angle to a maximum half-width of less than 5 Hz at 15°. Beyond this angle the half-width was roughly equal to or less than the half-width observed at 15°. This angular dependence may be due to the apparent narrowing of the view between chloroplasts because of their thickness, since the narrowest spacings produce the highest modulation frequencies. For example, a flat array of chloroplasts 3.8 μm thick and separated by 1.0 μm would completely obscure the view of particles traveling behind the chloroplasts for observation angles greater than 15°. Thus the width, form, and angular dependence of the low-frequency component are consistent with modulation of the scattering intensity by the chloroplast array.

If the low-frequency component of the heterodyne spectrum is the result of modulation by the chloroplasts, then it should not be present if the chloroplasts are removed. Local removal of the chloroplast array by photobleaching to produce a "window" has been described (1, 10, 20). Scattering from a window region 48 h after photobleaching gives a laser Doppler spectrum very similar to that obtained from a normal region of the cell, except that the intense low-frequency component is absent from the spectrum. Fig. 3 shows the spectrum of the scattered light from a window in an internodal cell of *Nitella*. The incident light of λ = 632.8 nm had a beam diameter of 50 μm. The scat-

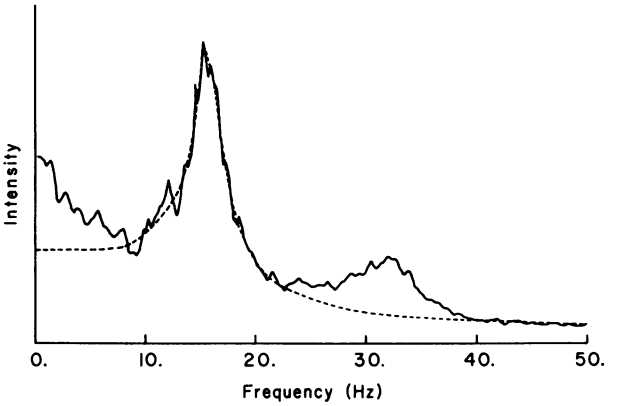

FIGURE 3 The spectrum of the scattered light collected from a window region of a *Nitella* internodal cell. Intense laser light bleaches the cell and induces the local detachment of the chloroplasts, producing a "window" region. Protoplasmic streaming viewed in a window region regains a frequency distribution characteristic of unbleached regions after 48 h. However, the intense modulation component centered at zero frequency is now absent. A new spectral component at twice the normal peak frequency is introduced that does not contribute to the velocity histogram and may be subtracted. An equal component centered at zero frequency arises and can also be subtracted. The dashed line represents these corrections and illustrates the frequency distribution of the scattered light from *Nitella* in the absence of modulation by the chloroplasts. A velocity distribution can be determined directly from this spectrum (see Fig. 6).

tering angle was 11.2° and the temperature was 20°C. A low scattering angle was chosen to avoid viewing the scattering region through unbleached regions to the side of the window. The low-intensity peak centered at zero frequency in Fig. 3 is directly attributable to a homodyne or "self-beat" component in the spectrum. This peak is the result of the diminution of heterodyne reference scattered light due to the removal of the stationary chloroplasts. Removal of this portion of the heterodyne reference scattered light also enhances the relative intensity of a peak occurring at twice the normal peak frequency as the result of "cross-beating" between the light scattered from the two opposite streaming directions in the cell. If the two directions of flow produce equal intensities of scattered light, then the cross-beat peak should have the same intensity as the self-beat peak centered at zero frequency. The amplitude of the self-beat peak can be estimated from the amplitude of the cross-beat peak and subtracted from the spectrum to give approximately the low-frequency intensity in the absence of both homodyne contributions and intensity modulations from the chloroplast array. The dashed line in Fig. 3 shows the spectrum corrected for these effects. We feel that this adjusted spectrum may be regarded as a velocity histogram for protoplasmic streaming in a *Nitella* internodal cell window. The remaining low frequencies are most likely the result of slow velocities and discontinuous motions in the protoplasm.

If the foregoing interpretations are correct, then light scattered at zero scattering angle from a window in an internodal cell should produce a spectrum with no intensity at frequencies other than zero. We have performed this experiment, with the result that only a very low-intensity spectrum can be detected. The width of this spectrum is narrower than the spectrum in Fig. 2*a* by a factor of two. We attribute this weaker spectrum to modulations of intensity produced by fluctuations in the number and sizes of scatterers in the beam.

Vacuolar motion in internodal cells of *Nitella* is difficult to measure with a light microscope because of the small number of visible inclusions in the vacuolar sap and the presence of the dense endoplasmic layer and cell periphery. We have found that the image resolution of phase contrast and Nomarski microscopic measurements of vacuolar motion are improved by observation through a photobleached window. A solution of the hydrodynamic equations for vacuolar motion has been formulated for *Chara braunii* by Pickard (23). The same analysis may be used for *Nitella flexilis* because of the great similarity of protoplasmic streaming in these two cells. The theoretical result for the velocity as a function of position 90° to the indifferent zones in the cell is, $V = (2v_m/\pi)$ arc tan $\{(2r/r_0)/[1 - (r/r_0)^2]\}$, where v_m is the maximum velocity at the boundary (endoplasm), r_0 is the radius of the cell interior, and r is the radial distance from the center. This equation was solved for a constant v_m between indifferent zones, consistent with our spatial measurements of the most probable streaming velocities in *Nitella* (20). A plot of the velocities measured by optical microscopy from the endoplasm at one side of the cell to the center of the vacuole is shown in Fig. 4, together with the least-squares fit to the theoretical flow equation. The radius of the cell interior was 120 μm and v_m was determined to be 65 μm/s. Velocities measured by focusing beneath the center of the cell have the same form as Fig. 4 but opposite sign.

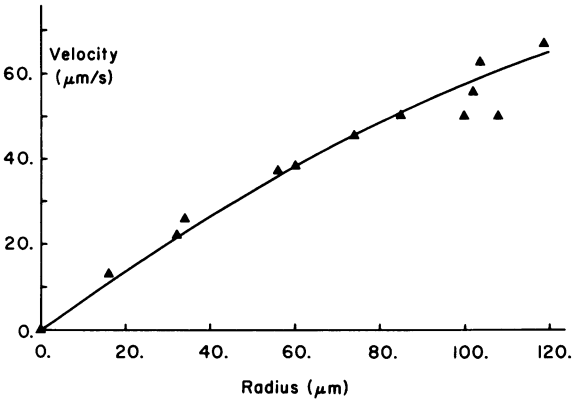

FIGURE 4 Velocity as a function of radius in an internodal cell of *Nitella*. The triangles are the vacuolar and endoplasmic velocities measured by light microscopy by viewing the cell interior through a window region halfway between the indifferent zones. Zero radius represents the center of the vacuole. The tonoplast, the membrane separating the vacuole and endoplasm, is in the region of approximately 100 μm radius. The line represents the least-squares fit of the theory to the measured velocities. The presence of large velocities inside and outside the tonoplast indicate that it is flowing along with the vacuolar sap and endoplasm in the cell interior.

The scatter of data points in the endoplasm (in the region of approximately 100–120-μm radius) is a result of sampling the distribution of endoplasmic velocities. Velocities on both sides of the tonoplast, the membrane separating the vacuole from the endoplasm lying at a radius of approximately 100 μm in Fig. 4, are roughly equal. Thus the tonoplast appears to be shearing like a simple fluid inside the cell. There must be considerable lateral shear on the tonoplast towards the indifferent zones since the protoplasmic velocities are of opposite sign when crossing the indifferent zones. It is difficult to reconcile the existence of any known membrane with the protoplasmic velocity distribution at this interface.

The velocities in the vacuole have magnitudes and orientations that should contribute to the intermediate and lower frequencies in the light scattering spectrum. However, orientation of the cell such that the laser beam impinges only on the edge of the cell results in Doppler frequency spectra essentially identical to the spectrum in Fig. 1 (20). This rules out vacuolar motion as an important source of intermediate and low-frequency intensity in the light scattering spectrum.

We have observed the frequency spectra from the streaming cells treated with ATP, cytochalasin B, and colchicine to determine the sequence of changes in the velocity distributions resulting from the effects of these agents. Cells were first monitored for 20 min to establish stable streaming velocities and then the medium surrounding the cells was changed to a medium containing the drug. Addition of ATP at a concentration of 10^{-2}M in artificial pond water to a normally streaming internodal cell of *Nitella* immediately reduced the streaming velocities by a factor of two. The frequency spectrum lacked a Doppler-shifted peak, indicating a large relative increase in the lower streaming velocities. Streaming ceased completely within 30 min. Addition of

10^{-3} M ATP to a normally streaming cell again increased the low-frequency contributions to the frequency spectrum, so that a Doppler-shifted peak was not observable. However, streaming was still apparent at the highest velocities normally observed. No noticeable effect on the light-scattering spectrum was produced by addition of 10^{-4} M ATP to normally streaming cells.

Addition to internodal cells of cytochalasin B (Aldrich Chemical Co., Inc., Milwaukee, Wis.), in concentrations of 1–50 μg/ml in artificial pond water containing 1% dimethyl sulfoxide, inhibited streaming. The course of the streaming inhibitions could be followed with the tracking circuit developed for measuring the position of Doppler-shifted peaks (19). The inhibition of streaming showed a nonlinear dosage dependence for the concentrations used (10–50 μg/ml) and a strong temperature dependence. Fig. 5 shows the changing streaming velocities for inhibition by 30 μg/ml cytochalasin B at 12°C and 21°C. The initial rates of velocity reduction differ by a factor of two. During inhibition, the Doppler-shifted peak was observed to move slowly to lower frequencies with no change in the shape of the spectrum.

Cells exposed to very low levels of cytochalasin B (such as 1 μg/ml) showed reduced streaming after many hours and a change in shape of the Doppler frequency spectrum. The intermediate and low-frequency intensity increased so that a Doppler-shifted peak was still apparent but with less than 20% more intensity than the intermediate frequencies.

The addition of colchicine at a concentration of 1% to internodal cells produced no immediate changes in the light-scattering spectra. After 24 h the streaming rate was reduced by approximately 10% but there were no changes in the shape of the spectra.

DISCUSSION

Recent studies of protoplasmic streaming in the *Characeae* have shown the interface between the ectoplasm and the endoplasm to be the locus for motive force generation

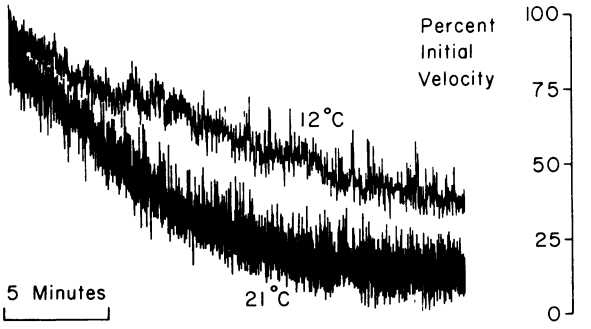

FIGURE 5 Inhibition of protoplasmic streaming in *Nitella flexilis* by cytochalasin B at different temperatures. The peak streaming velocity was monitored continuously by a special tracking circuit. The two curves show the response of the streaming to the addition of 30 μg/ml cytochalasin B at 12°C and 21°C. The initial rates of streaming decrease differ by approximately a factor of two.

(7, 9, 11, 12, 16, 21). Fibrils consisting of microfilaments present along the ectoplasm-endoplasm interface are aligned with the streaming (8–12, 21, 22). Organelle movement in the endoplasm has been observed along these fibrils (1, 9, 34). In addition to endoplasmic shearing along the ectoplasm-endoplasm interface, it has been suggested that propagating, bending waves of fibrils extending into the endoplasm from the cortical fibrils are a motive force for streaming (1).

In previous laser light-scattering studies we have shown that the endoplasm streams with a narrow distribution of velocities and without any appreciable diffusion relative to the transport velocity (20). The only uncertainty in interpretation of the Doppler spectra as velocity histograms was the determination of the source of the abundant low-frequency intensity. Our demonstration that the low-frequency intensity is attributable to the intensity modulation of scattered light by the chloroplast array allows us to construct a velocity histogram for streaming velocities of all magnitudes in a window region of *Nitella*. Since the light scattering spectra from window regions and unbleached regions are very similar except for the very low-frequency portion of the spectrum, we surmise that the velocity histogram obtained from correction of the frequency spectrum for homodyne effects in the window region will correspond closely to the velocity histogram for normal, unbleached regions of the cell. The result is represented in Fig. 6. Region I of the histogram shows a narrow distribution of streaming velocities that comprises approximately 60% of the scattering intensity. The peak velocity corresponds to the streaming velocity, which would be determined by visual observation. The peak velocity is a sensitive function of temperature and the distribution in region I narrows slightly with increasing temperature (20). Region II

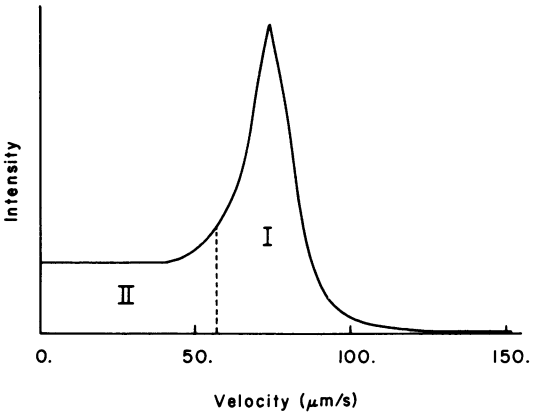

FIGURE 6 The velocity histogram for protoplasmic streaming in *Nitella flexilis*. This histogram is directly calculated from the dashed line shown in Fig. 3. The histogram is divided into two regions for the discussion in the text. Region I contains 60% of the intensity and corresponds to a narrow distribution of streaming velocities about the normally cited streaming velocity. Region II comprises the remaining 40% of the intensity. The velocities in region II are presumed to result primarily from discontinuous motions of endoplasmic particles along the ectoplasm-endoplasm interface.

represents the distribution of lower velocities and contains the remaining 40% of the intensity.

It is surprising that region II constitutes such a large fraction of the velocity histogram. We have shown that vacuolar motions do not contribute significantly to region II of the histogram. Therefore, most of the intensity in region II is from endoplasmic scatterers. We recognize three possible endoplasmic sources of velocities that can make major contributions to region II. The first possible source is the undulations of endoplasmic filaments reported by Allen (1). These undulations could constitute a fluctuating change in the refractive index of the local medium and hence contribute to the light-scattering spectrum. The frequencies of oscillation quoted by Allen are in the region of the low-frequency component of our spectra. However, these filaments are not large in comparison with other endoplasmic particles, and the magnitude of the refractive index change which they could induce must be considered small. Therefore, the undulation of endoplasmic filaments would not be expected to produce a significant intensity of scattered light by comparison with the scattering from endoplasmic particles, so we regard this possibility as remote. The second conceivable source is low velocities produced by shearing of the protoplasm along the ectoplasm-endoplasm interface. Electron micrographs have shown that the cortical fibrils are parallel to the streaming direction along the ectoplasm-endoplasm interface and are approximately 0.5 μm apart (14, 21). Between these fibrils there would be zones of low protoplasmic velocities. A third possible endoplasmic source of intensity in region II is discontinuous motion of scattering particles along the cortical fibrils. If motion of a particle is interrupted for short times by collisions with other particles or obstructions in the ectoplasm, then the spectrum will show lower-frequency components resulting from the averaging of the velocities over the experimental sampling time (1–4 s). Also, the contractile process along the fibrils may be inherently discontinuous due to structural features of the fibrils and fluctuations in the concentrations of chemicals and proteins necessary for contractility. Discontinuous motion of larger particles in the endoplasm near the ectoplasm-endoplasm interface has been reported previously (34), and we have confirmed this observation by optical microscopy using phase contrast and Nomarski optics. The ectoplasm-endoplasm interface is the most visible region of the protoplasm for inspection by light microscopy, and there has been no observation of a substantial fraction of particles along this interface continuously streaming at slower velocities. Therefore, we do not believe that continuous shear at the ectoplasm-endoplasm interface will account for the large fraction of velocities in region II. Discontinuous motions of protoplasmic particles near contractile fibrils have been observed by us and others, and these may be the major source of lower velocities.

The structure of the endoplasm-vacuolar interface poses an intriguing problem. The tonoplast has not been observed in electron micrographs, presumably because of difficulties in fixation of the protoplast. Electrophysiological measurements imply the existence of a membrane (27). Endoplasmic and vacuolar motions, on the other hand, continuously shear the tonoplast, and the velocities in Fig. 4 indicate that the shear is transmitted undiminished. Protoplasm drained from cut *Nitella* cells has been shown

to form a membrane from phospholipids and proteins present in the protoplasm (28). Fragments of tonoplast and plasmalemma may also be incorporated into this membrane. The capability of membrane formation by phospholipids and proteins present in the protoplasm suggests the possibility that the protoplasm may be capable of continuous repair of the tonoplast as it is being sheared by protoplasmic streaming along the indifferent zones.

ATP has been observed in experiments with *Chara* to detach endoplasmic organelles from the visible cortical fibrils (34). These fibrils have been shown to contain actin, which binds rabbit heavy meromyosin with polarity, suggesting unidirectional motion of particles and organelles along the fibrils (15, 22, 33). Rabbit myosin has been shown to dissociate from *Chara* actin when ATP is added (33). ATP has been found to stimulate protoplasmic streaming velocities by 15% in *Nitella flexilis* for concentrations of 10^{-3} M ATP in the surrounding medium (26). We observed no substantial change in the maximum streaming velocities when ATP was added in this concentration. However, there is a large increase in the relative amount of lower velocities in the velocity distribution. Addition of 10^{-2} M ATP reduces the maximum steaming velocities by a factor of two, with a similar change in the shape of the velocity distribution. These concentrations of ATP exceed estimates of approximately 4×10^{-4} M ATP for the normal ATP concentration in *Nitella* endoplasm (12). The observed increase in lower-frequency contributions to the frequency spectrum suggest an increase of discontinuous motion of endoplasmic particles and organelles along the fibrils as a result of particle-fibril dissociation by ATP.

Cytochalasin-binding studies in a variety of cells have indicated sites of different binding affinity (17, 29). It has been suggested that low affinity sites may be the important binding sites for inhibition of motility in biological systems (17, 29). Organelle movement along actin-containing fibrils may be an indication of the presence of some myosinlike protein in the endoplasm. Inhibition by cytochalasin B may involve inactivation of the contractile apparatus by binding with endoplasmic myosin (24, 29). Cytochalasin B has been observed to halt organelle motion along visible fibrils in *Chara* and to prevent the release of organelles from the fibrils by ATP (34). Our experiments indicate that the protoplasm continues to stream with a narrow distribution of velocities for high cytochalasin B dosages, with the velocity decreasing as a function of time. However, at low dosages (1 μg/ml) the shape of the frequency spectrum changes many hours after addition of the drug. A Doppler-shifted peak is still present but with only moderately (<20%) greater intensity than the intermediate frequencies in the spectrum. Thus there is a relative increase of lower streaming velocities in the velocity distribution. Uptake studies with a variety of cells have shown that cytochalasin partitions itself between the medium and the cells. This suggests a noncovalent binding of the drug. The observation of the increase in lower frequencies in the cells treated with low levels of cytochalasin B may reflect a different partitioning of the drug in the cell. It has been suggested that use of 0.5–1 μg/ml concentrations of cytochalasin B may be ineffective for the saturation of binding sites in cells in microchambers (13), and our results would be consistent with that suggestion.

Colchicine is known to bring about the disruption of cytoplasmic microtubules (2, 3, 6, 25, 30, 31). Microtubules in *Nitella* have been observed between the plasmalemma and the cell wall at an angle to the streaming and out of contact with the streaming endoplasm (21). The lack of effect of colchicine on protoplasmic streaming in *Nitella* has been reported previously (4, 13). Experiments using both colchicine and cytochalasin B to inhibit protoplasmic streaming in *Nitella* show the same inhibition kinetics as colchicine-free controls (4). We observe no changes in the frequency spectrum several hours after addition of very high concentrations of colchicine. Thus, we have shown that colchicine has no detectable effect on any component of the distribution of velocities in *Nitella*.

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