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The Endogenous Difference in the Rates of Acropetal and Basipetal Cytoplasmic Streaming in *Chara* Rhizoids is Enhanced by Gravity

Z. HEJNOWICZ[1], BRIGITTE BUCHEN*, and A. SIEVERS

Botanical Institute, University of Bonn

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Summary

Measurements of cytoplasmic streaming in *Chara* rhizoids were made by a streak-photography method combined with dark-field illumination. The velocity of cytoplasmic streaming in the acropetal direction was faster than in the basipetal direction. The difference in the streaming velocities in both morphological directions was apparently due to endogenous forces. In addition to this, a small difference attributable to gravity was superimposed if the rhizoid was oriented parallel to the gravity vector. The difference in the endogenous forces underlying the oppositely directed streams may be as high as about 12-fold the force imposed by gravity but, on average, it is about 5-fold the gravity force. In the normal vertical position of the rhizoid, this endogenously generated difference is enhanced by the gravity effect. In contrast to the variability of streaming rate due to endogenous forces, the effect of the gravity force is relatively uniform. The difference between acropetal and basipetal streaming velocities is perpetuated over the whole range of lowered velocities after treatment with cytochalasin B. After prolonged incubation in cytochalasin B, the basipetal streaming stops earlier than the acropetal streaming. A difference in the length of filaments on both sides of the streaming machinery in rhizoids is proposed as the structural basis for the difference in velocities.

Keywords: *Chara* rhizoid; CB action; Cytoplasmic streaming; Gravity; Polarity.

1. Introduction

Since the observations of EWART (1903) it has been known that a difference exists in the rates of acropetal and basipetal cytoplasmic streaming in plant cells (BOTTELIER 1934, HAYASHI 1957). The factor responsible for this difference is considered to be the force of gravity acting on the opposed streaming directions (KAMIYA 1959). Yet comparing the data obtained from cells in normal vertical, inverse vertical and horizontal orientations (EWART 1903, BOTTELIER 1934), it seems doubtful that the differences in velocities are due only to the gravity force. Therefore, we have tried to clarify these phenomena and the factors involved.

It is known that cytoplasmic streaming depends on the activity of the microfilament system (for reviews, see WILLIAMSON 1980, ALLEN 1981, KAMIYA 1981). The polarity of the microfilaments determines the direction of streaming (KERSEY *et al.* 1976). Cytochalasin B (CB) impairs this activity (WILLIAMSON 1975, MACLEAN-FLETCHER and POLLARD 1980, BROWN and SPUDICH 1981, NOTHNAGEL *et al.* 1981). Hence a difference in acropetal and basipetal streaming implies that the two parts of the underlying microfilament system, which differ in polar orientation of the filaments with respect to the cell axis, may not be equal and the system as a whole may be polar. Since the microfilament system is a part of the cytoskeleton, the total polarity of the system

[1] Visiting Professor on a grant from Deutsche Forschungsgemeinschaft. Permanent address: Department of Biophysics and Cell Biology, Silesian University, ul. Jagiellonsky 28, P-40-032 Katowice, Poland.

* Correspondence and Reprints: Botanical Institute, University of Bonn, Venusbergweg 22, D-5300 Bonn 1, Federal Republic of Germany.

represents an interesting example of cytoskeleton polarity worthy of study.

2. Material and Methods

Rhizoids of *Chara contraria* Kütz. were obtained by the method described earlier (HEJNOWICZ and SIEVERS 1981). For recording, the rhizoids were prepared in two different ways: (i) a node with rhizoids was mounted in a chamber allowing controlled flow of solution (HEJNOWICZ and SIEVERS 1981); (ii) a small channel was built on a slide using two parallel strips of cellophane or cover glasses. An internode with nodal cells bearing rhizoids was put in a drop of nutrient solution on the slide, the rhizoids lying in the channel. The rhizoids and the rest of the thallus were covered by separate cover glasses. During recording in the horizontal position, the solution was either stationary or a flow was achieved by supplying drops at the rhizoid tip and absorbing the solution with filter paper at the nodal cells. In the case of recording in the vertical position, the cover glasses were fastened by lanoline (Vaselin DAB 8, Engelhard, Frankfurt/M., FRG) and the end of a thin plastic tube providing the solution was attached to the top of the chamber. Thus, the solution slowly flowed through the channel containing the rhizoids.

The experiments were made at 24 ± 1 °C. Even prolonged and strong lighting of the rhizoid in a stationary solution caused a temperature enhancement of 1 °C only. To limit the effects of temperature changes during the experiments, the light intensity was immediately reduced after the exposure normally lasting 5 s.

The rhizoids were observed either on a horizontal stage of a Zeiss photomicroscope III or on a vertically oriented stage of a horizontal microscope which could be rotated around its horizontally oriented axis.

The vertical position of a recorded rhizoid was either normal, *i.e.*, with the apex downward (Fig. 1 *a*), or inverted, *i.e.*, with the apex directed upward.

The recording was by means of streak photography (HEJNOWICZ and SIEVERS 1981). The image of the rhizoid was of the type "dark-field". In case of the use of 10 × or 16 × objectives, the condenser was shifted

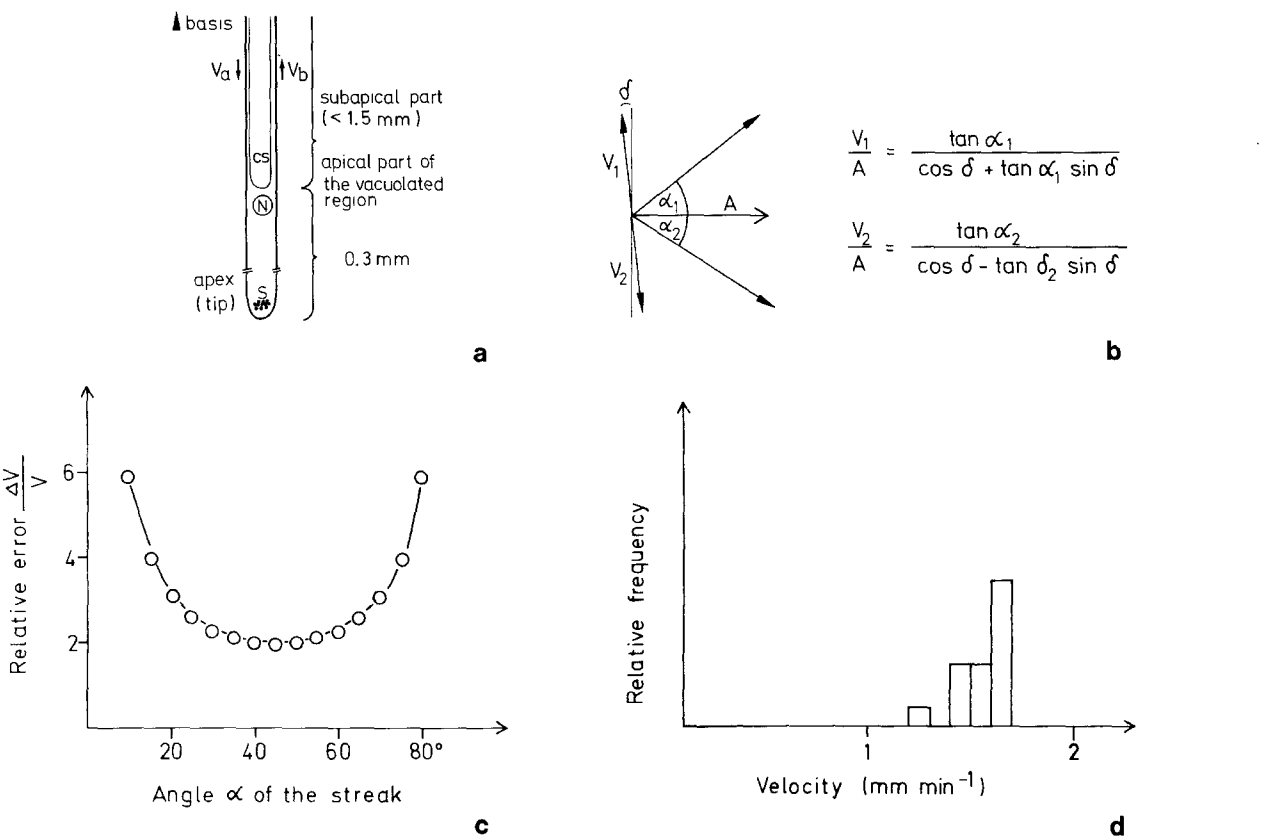


Fig. 1 *a*. Scheme of a *Chara* rhizoid in normal vertical orientation. The vacuolated part (*CS* cell sap) about 0.3 mm basal to the rhizoid tip is surrounded by the streaming endoplasmic bands. The direction of streaming is indicated by $V_a\downarrow$ and $V_b\uparrow$ respectively. Usually, the streaming velocities were recorded within the subapical zone of the vacuolated region (0.3 to 1.5 mm basal to the rhizoid tip). The dimensions of the endoplasm do not correspond to the scale (diameter of the rhizoid: 30 µm). *N* nucleus; *S* statoliths

Fig. 1 *b*. Relationship between the velocities of streaming in opposite directions V_1 and V_2, the tangents of the angles α_1 and α_2, and the angle δ between the direction of streaming and the perpendicular to the direction of film movement A

Fig. 1 *c*. Relative error in evaluation of streaming velocity on the basis of the angle α between the streak and the horizontal, as a function of the angle α

Fig. 1 *d*. Histogram showing the distribution of streaming velocity in one direction observed in a window 100 µm·3 s on a streak photograph (based on 14 streaks in the window)

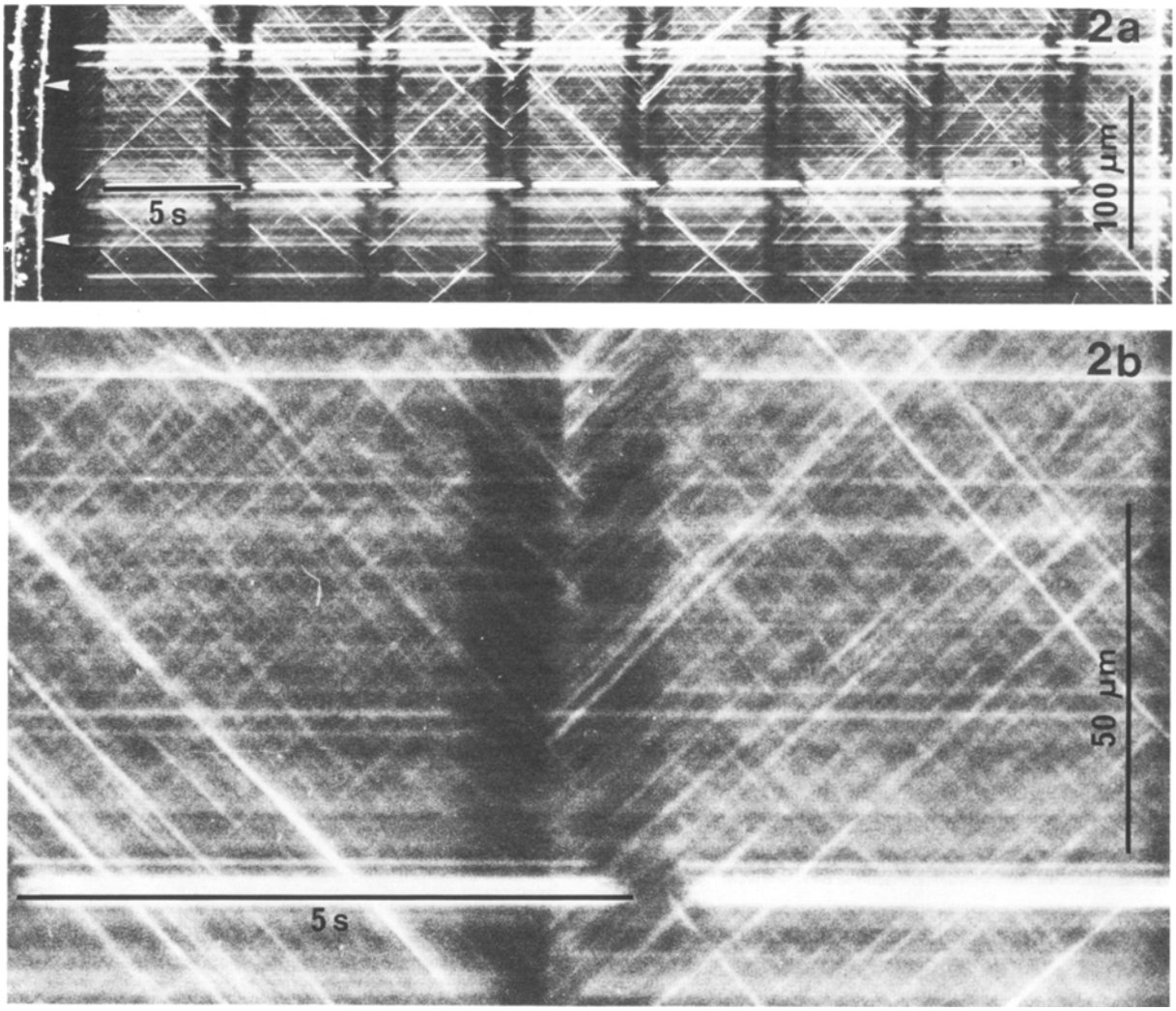


Fig. 2 *a*. Streak photography of a rhizoid in the horizontal position (objective 10×; time of exposure for each photograph, 5 seconds; interval between two photographs, 1 minute). At the beginning, the photographic outline of the recorded rhizoid segment. The window for evaluation of V_a and V_b is indicated by arrowheads. *b* 2 successive streak photographs at higher magnification

from the axial position until the cytoplasmic particles were bright, contrasting with the cell wall and the background. Using the 100× objective, the image was obtained by means of a dark-field condenser. The image was focused on high-speed film through a slit 1.5–3.0 mm wide in a plate positioned just in front of the film. The streaming was adjusted parallel to the slit. During exposure, the film moved at a constant velocity in the direction perpendicular to the slit, *i.e.*, perpendicular to the streaming. The bright elements of the image produced streaks on the film. In order to record the streaming simultaneously in both directions, we chose those rhizoids which showed, in the focus plane, the two oppositely directed streams of endoplasm on either side of the vacuole. The zones between these streams (above and below the vacuole) were out of focus.

The images of both streams were adjusted to be within the slit. Thus, on a single photograph there were streaks representing both acropetal and basipetal streaming (Figs. 2 *a* and *b*). A single exposure generally lasted 5 seconds. The intervals between the exposures depended on the rate of change of velocity. When the state was steady, this interval was usually 1 minute.

The higher the velocity of a moving particle, the wider is the angle α between its streak on the film and the direction of film movement which will be called horizontal for convenience. If the streaming is perpendicular to horizontal (this streaming will be termed vertical), the tangents of α for acropetal and basipetal streaks are proportional to the streaming velocities: $V = A \tan \alpha$, where V and A are the streaming and film velocity, respectively. However, if the direction of streaming deviates from the vertical by angle δ, then tan α for particles moving in one direction is increased while tan α for particles moving in the opposite direction is decreased. Fig. 1 *b* shows the relationship between V and δ. The method is rather sensitive to the angle δ. The accuracy of making the angle δ as small as possible in our streak photographs was not worse than 2°.

Another source of error in evaluation of streaming velocity lies in the measurement of the angle α. To find the minimum of relative error

$$\frac{\Delta V}{V} = \frac{dV}{V}$$ let us introduce $V = A \tan \alpha$ and $dV = A \frac{d\alpha}{\cos^2 \alpha} + \tan \alpha dA$

into the last equation. Assuming that A is constant (the velocity of the

film movement), $dA = 0$, we obtain $\frac{dV}{V} = \frac{d\alpha}{\sin\alpha\cos\alpha}$ which has a minimum for $\alpha = 45°$. The dependence of the relative error upon α is shown in Fig. 1 *c*. The most appropriate angle of streaks is in the range of 30–60°, thus the velocity A was chosen so that α was in this range.

The stock solution of cytochalasin B (CB; EGA-Chemie, Steinheim, FRG) of 100 µg ml^{-1} in 1% dimethylsulfoxide (DMSO; Serva, Heidelberg, FRG) was diluted with culture medium (FORSBERG 1965) to the concentration used, 5–30 µg ml^{-1}. Stock solution of N-ethylmaleimide (NEM; Serva, Heidelberg, FRG) at 10 µg ml^{-1} in distilled water was diluted with medium to 1–3 µg ml^{-1}.

3. Results

3.1. General Characteristics of Cytoplasmic Streaming

The majority of particles were located within opposite bands of streaming endoplasm. In the intermediate zones between these bands, the particle density was much lower; these zones were relatively wide, their width amounting to ¼–⅓ of the band width. In general, the particles in the intermediate zones showed only Brownian movement and no continuous streaming.

Within the streaming bands, the streak photographs of moving particles indicated little variation in the inclination of streaks. This means that there was little variation in the velocity vector, *i.e.*, both with respect to the magnitude and the instantaneous direction of velocity. Some particles in the bands were translocated very slowly or even stopped transiently but such events concerned single particles and not groups of them.

The average velocity in the streaming endoplasmic bands varied from 1.23 to 2.91 mm min^{-1} with the mean standard error of 0.1 mm min^{-1}. These data were taken from 23 recorded rhizoids with the average velocity for each rhizoid taken from 8 or more streak photographs (*cf.* Fig. 2 *a*). For one rhizoid with a large temporal variation of velocity (0.68 to 1.78 mm min^{-1}) the mean velocity was not calculated.

3.2. Velocity of Acropetal and Basipetal Streaming

A typical streak photograph of a rhizoid is shown in Fig. 2 *a*. The streaks are mostly parallel though not quite straight due to some spatial and temporal variation of the individual particle velocity. It is thus important to choose an appropriate "window" to observe the streaks. We have chosen the window 100 µm·3 s and considered the streaks running through this window. The typical distribution of the velocities corresponding to streaks running through such a window is shown in Fig. 1 *d*. There is a sharp cut of the velocity range in the higher levels. Thus, for characterizing the streaming velocity in a given position at a certain time we took the steepest streaks at a considered site.

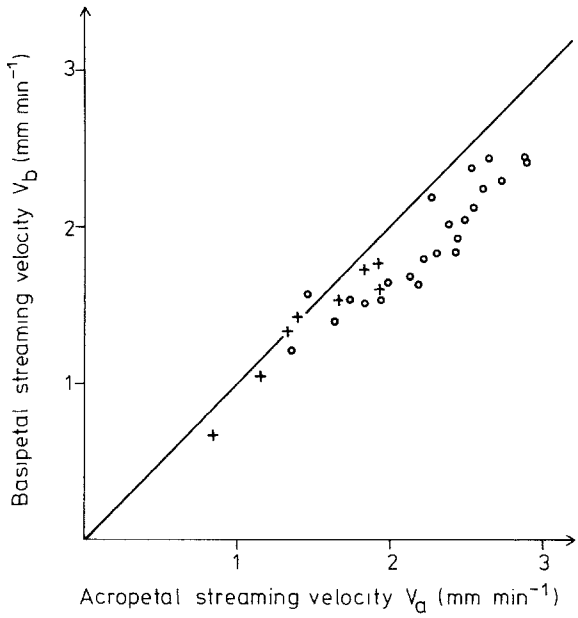


Fig. 3. Relationship between acropetal and basipetal streaming velocities in rhizoids in the horizontal position. Circles: averages for 23 rhizoids; each circle represents mean velocities of a single rhizoid in the vacuolated region extending to about 1.8 mm from the cell tip. Crosses: individual velocities from successive photographs of one rhizoid in which the velocity increased with time

3.2.1. Horizontally Oriented Rhizoids

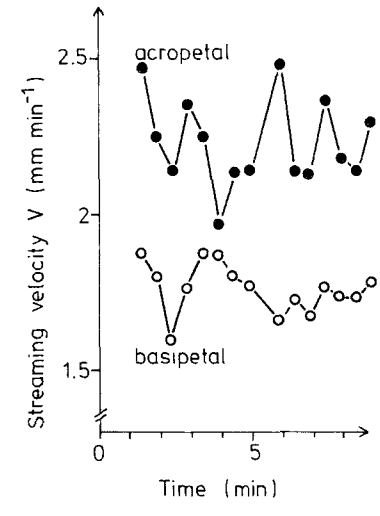
Both streaming velocities—acropetal termed V_a and basipetal termed V_b—were simultaneously recorded at one site of the cell. With respect to the whole rhizoid axis about 10 mm long, this site was normally chosen within the subapical part of the vacuolated region extending from 0.3 mm to about 1.5 mm behind the rhizoid tip (Fig. 1 *a*). Usually, the velocities V_a and V_b are different, V_a being higher than V_b. The total averages for V_a and V_b were 2.25 mm min^{-1} and 1.93 mm min^{-1} respectively. The average difference V_a–V_b was about 0.32 ± 0.18 mm min^{-1}. The relationship between the average velocities, each pair at the same site of the rhizoid, is shown in Fig. 3. The general tendency of the points to be accumulated below the diagonal (*i.e.*, equal velocity in both directions) is obvious, though in some rhizoids the difference may not be significant.

There may be temporal variation of the streaming velocity at a single site in a rhizoid as well as spatial variation of velocity along the rhizoid axis at the same

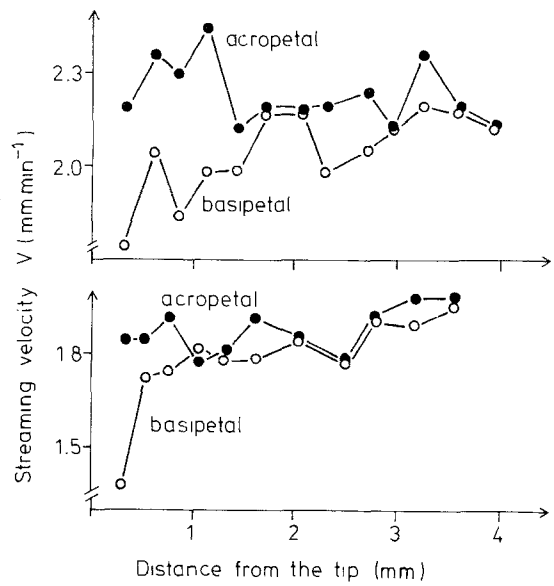
or nearly the same time. A typical temporal variation of V_a and V_b recorded at the same site of the cell about 0.7 mm from the tip is shown in Fig. 4 *a*. Such a temporal variation was not often closely correlated for the velocities in opposite directions. However, in the case of an obvious tendency to increase or decrease the velocity, the same tendency occurred for both velocities (crosses in Fig. 3).

It was tested whether the difference between V_a and V_b occurs along the whole rhizoid or is limited to the subapical part of the vacuolated region normally recorded. Fig. 4 *b* shows the spatial variation of the velocity with recording of successive zones about 0.3 mm in successive intervals of time for 2 rhizoids. Based on 5 examples, it seems that the difference between the velocities in opposite directions is more pronounced in the subapical part of the vacuolated region in the rhizoid.

In spite of the fact that V_a and V_b are different, an accumulation of endoplasm at the apical part of the vacuolated region was never observed (except after CB treatment, see p. 13). In growing rhizoids, the distance between the apical end of the vacuole and the rhizoid tip is constant, namely 0.3 mm (Fig. 1 *a*). As a consequence, the cross-section of the two opposite streaming bands must be different. It was not possible to measure the area of the cross-section through the stream (the latitudinal dimension of endoplasmic layer and the thickness in other planes than the focus plane). In the focus plane, however, the basipetal stream was obviously wider.



a



b

Fig. 4 *a*. Typical temporal variation of streaming velocities in a rhizoid about 0.7 mm from the cell apex with pronounced difference between both velocities. *b* Spatial variation of streaming velocities in 2 rhizoids

3.2.2. Vertically Oriented Rhizoids

After taking 4 streak photographs at 30-second intervals for each of 22 rhizoids in the normal vertical position, each rhizoid was inverted and 4 streak photographs were made in this position. For 6 rhizoids, this procedure was repeated by tilting the rhizoid back in the normal vertical position.

The relationship between acropetal and basipetal velocities in normal and in inverted positions is shown in Fig. 5. The relationship was variable but the tendency for $V_a > V_b$ is obvious. After inversion, too, the velocity of acropetal streaming was usually higher than that of basipetal streaming. The difference $V_a - V_b$, in the normal position, varied from 0.08 to 0.69 mm min^{-1}, the mean difference being 0.33 ± 0.14 mm min^{-1}. The corresponding values for the inverted position were -0.12 to 0.59 mm min^{-1} and averaged 0.19 ± 0.18 mm min^{-1}. The difference between the velocities was thus smaller in the inverted position. This indicates that there was an effect of gravity on the streaming velocities. Nevertheless, even in the inverted position, V_a was higher than V_b in the majority of the rhizoids, *i.e.*, the upward streaming was faster. This means that there is a tendency for a higher velocity of acropetal streaming independent of gravity.

The velocities due to endogenous forces acting in basal and apical directions are denoted as V and V + X, respectively, and the velocity due to gravity (buoyant force) as G. The latter is the velocity of movement of the

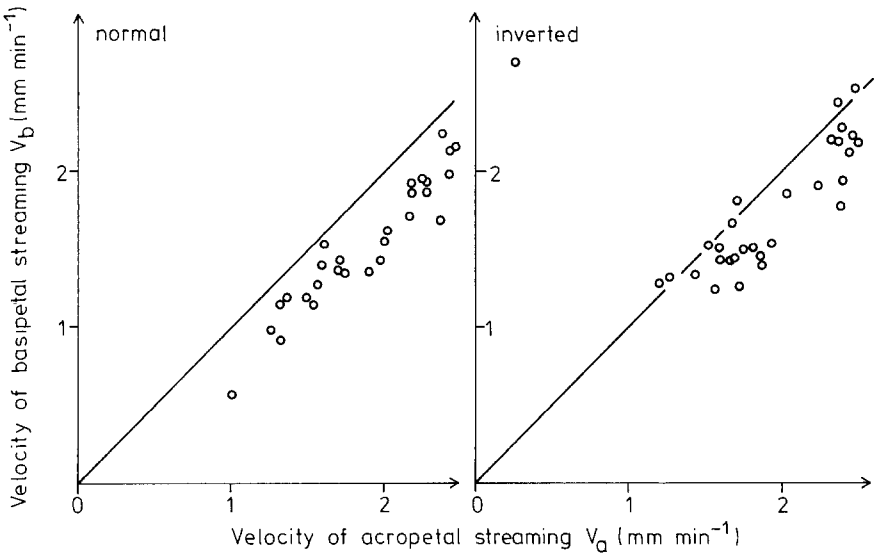


Fig. 5. Relationship between acropetal and basipetal streaming velocities in normal and inverted vertical position of rhizoids (mean values)

endoplasmic layer in spatial coordinates due to the slightly higher density of the endoplasm compared with that of the cell sap. X is the difference between the velocities in both directions due to endogenous forces. In the normal vertical position of the rhizoid, we have

$$V_a = V + X + G$$

and

$$V_b = V - G.$$

In the inverted vertical position we have

$$V_a = V + X - G$$

and

$$V_b = V + G.$$

Differences between the observed velocities in the normal and inverted positions are thus

$$V_a - V_b = X + 2G$$

and

$$V_a - V_b = X - 2G, \text{ respectively.}$$

Introducing the average values for the difference in velocities we have

$$X + 2G = 0.33\,\text{mm min}^{-1}$$

and

$$X - 2G = 0.19\,\text{mm min}^{-1}.$$

From subtracting and summing we obtain

$$4G = 0.14\,\text{mm min}^{-1}$$

and

$$2X = 0.52\,\text{mm min}^{-1}, \text{ respectively.}$$

Thus,

$$G = 0.04\,\text{mm min}^{-1}$$

and

$$X = 0.26\,\text{mm min}^{-1}.$$

These values have been obtained from averages for ΔV. Thus we should consider them only as rough approximations. It is tempting to obtain more reliable values from data for individual rhizoids. Yet an individual rhizoid should be used for calculation of G and X only if it does not show temporal changes of streaming velocity. In many rhizoids this condition was not fulfilled. Yet if the change of velocity were a consequence of the gravity effect only, the change in V_a and V_b should proceed in an opposite direction when changing the orientation of the rhizoid in the vertical position. Therefore, 8 rhizoids which fulfilled the latter condition, were used for individual calculations. For them, X varied from 0.02 to 0.50 mm min^{-1} with a mean value of 0.23 ± 0.17 mm min^{-1}, while G varied from 0.04 to 0.09 mm min^{-1} with a mean of 0.06 ± 0.01 mm min^{-1}. Thus, the difference between the acropetal and basipetal velocities due to endogenous forces, *i.e.*, X, varied widely in individual rhizoids. However, the velocity contribution due to gravity was relatively uniform.

There are good physical reasons for assuming that G is proportional to the gravity (buoyant) force and, in general, the velocity of streaming is proportional to the force acting on a particle. If we denote the gravity force corresponding to G as F_g, we can express the force causing movement of a particle in term of F_g. The force corresponding to the mean velocity 1.9 mm min^{-1} can be estimated as 32-fold F_g. The difference between the acropetal and basipetal forces underlying the velocity difference (which varied from -0.12 to 0.69 mm min^{-1} in different rhizoids ($n = 81$) with an average of 0.28 mm min^{-1}) can be estimated as ranging from -2 to 12-fold F_g with the mean being 5-fold F_g. Thus, in a rhizoid in the normal vertical position, the motive force acting acropetally is, on average, stronger than that

acting basipetally for 7-fold F_g (force corresponding to X + 2G).

3.2.3. Rhizoids Treated with CB

The streak photographs of rhizoids treated with CB show the typical decrease of streaming velocity after application of the drug (Fig. 6). CB exerts a characteristic effect on the streaming in the two morphological directions: the basipetal streaming stops earlier. Thus, the endoplasm accumulated in the apical part of the vacuolated region. We studied the effect of CB on rhizoids in horizontal, normal and inverted vertical positions. Thus, if there were a slower basipetal (downward) than acropetal (upward) streaming in the inverted position, an obvious difference in endogenous underlying streaming forces had to be involved. A typical time-course of changing the streaming velocity after application of CB is shown in Fig. 7. After treatment with 10 µg ml^{-1} CB, the velocity for acropetal streaming was always higher than that for basipetal streaming. There is a constant factor in the difference between the two velocities partially inhibited with CB (Fig. 8). After prolonged incubation, basipetal streaming stopped while acropetal streaming was still continuing.

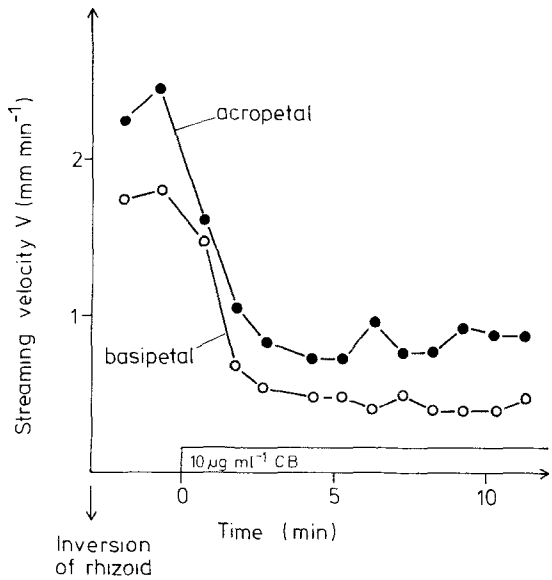


Fig. 7. Effect of 10 µg ml^{-1} CB on the streaming velocities in both directions in an inverted rhizoid

3.2.4. Rhizoids Treated with N-Ethylmaleimide (NEM)

NEM is known as an inhibitor of F-actin-activated myosin-ATPase (YAMAGUCHI *et al.* 1973). The action of NEM on the actomyosin system is quite different from that exerted by CB (CHEN and KAMIYA 1975, NAGAI and

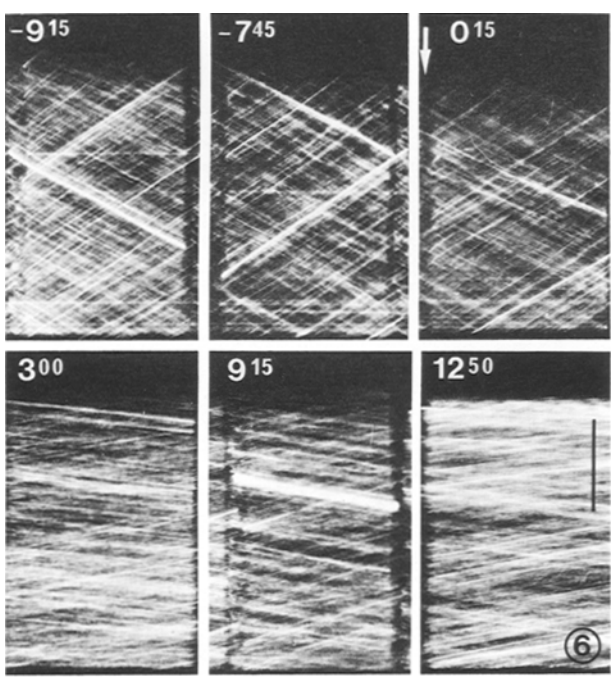


Fig. 6. Streak photographs of a rhizoid in the horizontal position before and after CB treatment (5 µg ml^{-1}). Arrow: CB application; the time (minutes, seconds) in respect to CB application (t = 0) is given on the single exposure. Bar = 100 µm

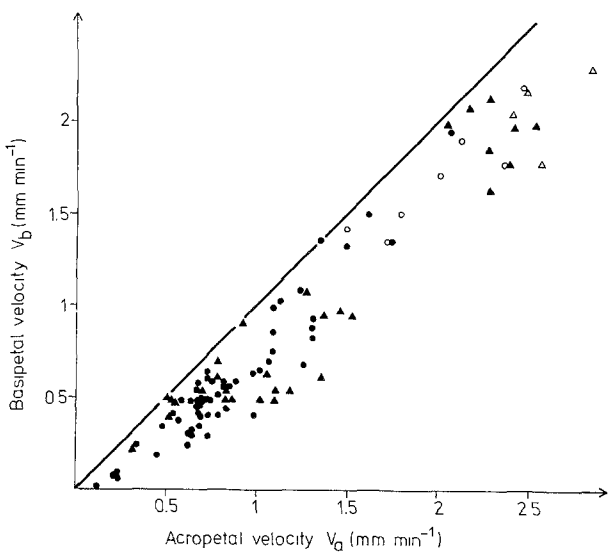


Fig. 8. Relationship between acropetal and basipetal streaming velocities in horizontal and in inverted vertical positions of the rhizoids before and after treatment with CB (30 µg ml^{-1}). △ mean velocity (based on a series of streak photographs) of a rhizoid in the horizontal position before CB application, ▲ single velocity (from a single streak photograph) of a rhizoid in the horizontal position after CB application, ○ mean velocity in the inverted position before CB treatment, ● single velocity in the inverted position after CB treatment

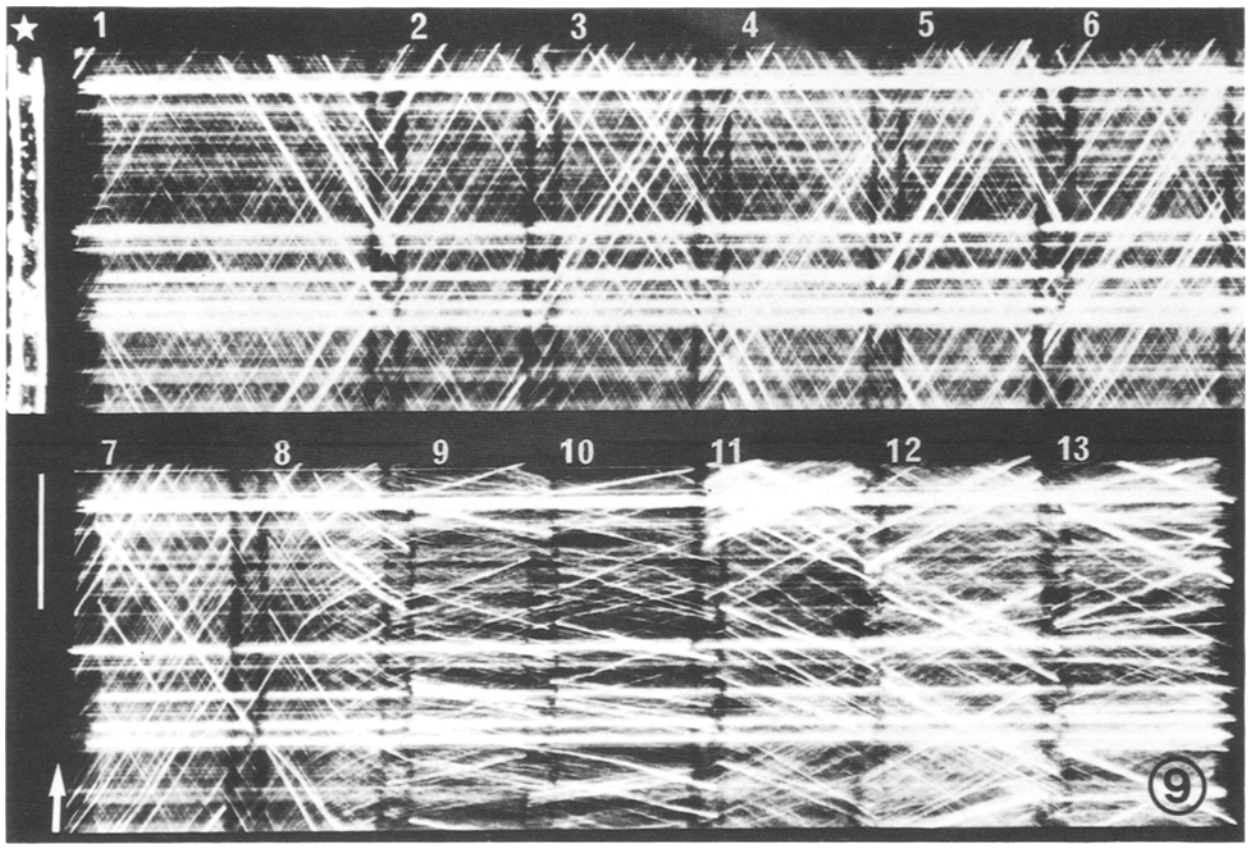


Fig. 9. Streak photography of a rhizoid in the horizontal position about 1 mm from the cell tip before (*1–6*) and after (*7–13*) application of 2 μg ml^{-1} NEM. * outline of the rhizoid segment, ↑ NEM application; photo *7* taken 15 seconds, photo *8* taken 32 seconds after NEM application. Bar = 100 μm

KAMIYA 1977). Rhizoids in the horizontal position were treated with 1–3 μg ml^{-1} NEM in nutrient solution. During treatment, severe morphological abnormalities could be observed which progressed while the streaming velocity decreased. Many particles appeared in the intermediate zones and vesicles and particles were seen in the vacuole. The endoplasmic band which normally flows as a whole along the ectoplasm lost its integrity during NEM treatment. This was reflected in an increasing variation of streak inclination (Fig. 9). Even if only the steepest streaks are taken into account for calculation of the velocity, there was a much wider spatial and temporal variation than in untreated rhizoids (Fig. 9, compare 2*a*), or in rhizoids treated with CB (compare Fig. 6). During approximately the first 100 seconds, no apparent effect on the streaming velocity could be observed. Thereafter, however, the slowing down of the velocity progressed rapidly and a low velocity was achieved in a few minutes. Later on, some recovery occurred but ultimately the streaming stopped. The low velocity level depended on the NEM

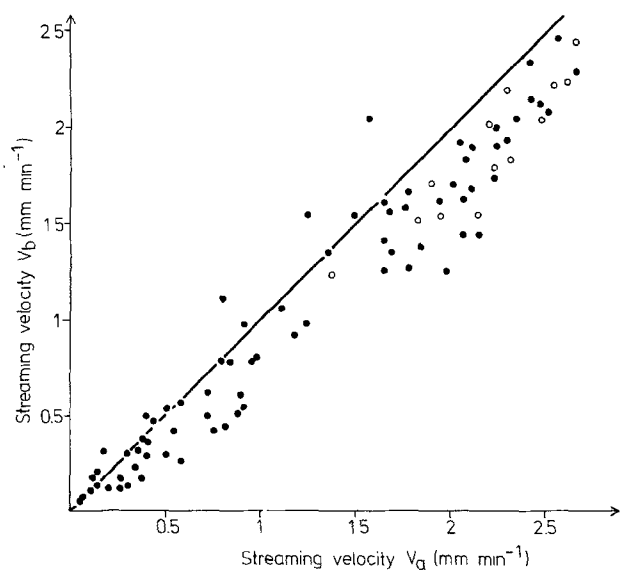


Fig. 10. Relationship between streaming velocities before and during NEM treatment (1–3 μg ml^{-1}). ○ average velocities before treatment with the drug, ● individual velocities from single streak photographs after treatment with NEM

concentration. The relationship between the velocities in both directions during NEM application is shown in Fig. 10. The dispersion of the points is wider than before treatment or than in CB-treated rhizoids (compare Fig. 8). In general, we did not observe a differential stopping of basipetal and acropetal streaming, as was the case in the experiments with CB.

4. Discussion

Protoplasmic streaming velocities may be determined by measuring the time taken for a distinct particle to travel a certain distance (HAYASHI 1957, CHEN 1973, NAGAI and KAMIYA 1977), by laser-light scattering (MUSTACICH and WARE 1976, SATTELLE and BUCHAN 1976) or by means of cinematography (KOOP and KIERMAYER 1980) and video recording (SEAGULL and HEATH 1980). The streak-photography method used in this study is a combination of prolonged-exposure photography of bright objects on a dark background with the "shadow imprinting method" of KAMIYA (1950). This method allows us to recognize simultaneously and at one single location the differences in streaming velocities in two opposite directions parallel to the cell axis of rhizoids. Hence, the interference of possible fluctuations in time and space of both the acropetal and basipetal streaming rates, which makes an accurate comparison of the two speeds impossible, could be eliminated. This enabled us to quantify both the endogenous and the gravity-induced differences in the streaming velocities. We also could recognize characteristic effects of cytochalasin B on the acropetal and basipetal streaming which may shed light on the mechanism of CB action.

4.1. Effect of Gravity

In a *Chara* rhizoid, the streaming velocity is higher in the acropetal than in the basipetal direction. This is shown for the normal vertical orientation of the rhizoid as well as for the horizontal and inverted vertical position (Figs. 3, 4 *a*, and 5). This means that there is an endogenously based difference in streaming velocities with the speed towards the tip being faster. Upon this endogenous streaming force, the gravity force is superposed.

The long-known difference in the rate of streaming in opposite directions was considered to be based on the gravity force acting on the endoplasm which is slightly denser than the cell sap (KAMIYA 1959, KESSLER 1979). In his study on the effect of gravity on green cells of *Chara*, HAYASHI (1957) found that the ratio of the original streaming velocity to the absolute amount of increasing or decreasing of velocity due to gravity is about 50. The acceleration in centrifugal experiments necessary to stop the centripetal streaming is also about 40 **g** (gravitational acceleration $\mathbf{g} = 9.81\ \mathrm{m\ s^{-2}}$). Thus, the streaming of the endoplasm has in the green Characean cells a balance-acceleration of about 40–50 **g**. A similar value (30 **g**) was reported for *Nitella* leaf cells (BRECKHEIMER-BEYRICH 1949).

According to HAYASHI (1957), the minute particles which are located near the ectoplasmic layer move independently of gravity. This observation cannot be supported by studies on the rhizoids. In the normal state, the endoplasm streams as a unit. There may be a local (temporally or spatially) nonconforming velocity of a particle. However, this phenomenon is not related to the position of the particle within the endoplasmic layer. Also KAMIYA and KURODA (1958) pointed to the sliding of this layer as a whole down over the ectoplasm. This may be clearly seen after centrifugation (especially in the rhizoids). The laser-light studies of *Chara* and *Nitella* internodal cells lead to similar conclusions; they show only a small range of deviation of velocities for the streaming particles (MUSTACICH and WARE 1976, SATTELLE and BUCHAN 1976). Such behaviour is in accordance with the hydrodynamic model of viscous coupling between motile myosin and endoplasm in Characean cells (NOTHNAGEL and WEBB 1982). This model is based on the concept that myosin is attached to a (fibrous/membranous) network in the endoplasm. The myosin slides along the actin bundles and the whole endoplasm is pulled forward. The structural basis for such an integrity of the endoplasm in internodal *Chara* cells is probably provided by filaments attached to protuberances present on organelles (NAGAI and HAYAMA 1979) or associated with the endoplasmic reticulum (WILLIAMSON 1979). It may also be provided by the three-dimensional network of filaments in the endoplasm demonstrated by rapid freeze-fracture and deep-etching techniques (ALLEN 1980). Yet more information is needed on the chemical nature of the filamentous network and its interaction with organelles to clarify the structural basis for the observed streaming phenomena.

If the endoplasmic layer slides down over the ectoplasm as a whole the gravity force acting on the layer can be calculated from the simple formula $F_g = Ad\,(D_{end}—D_{cs})\,\mathbf{g}$, where A is the surface of the endoplasmic layer, d is the thickness of the layer, D_{end} and D_{cs} are the densities of the endoplasm and the cell sap, respectively. This formula has been used by KAMIYA and KURODA

(1958) for calculation of the motive force using the value of the balance acceleration instead of **g**. The thickness of the endoplasmic layer in a rhizoid is about 5 μm. Taking the difference in densities of the protoplasm and the cell sap in the rhizoids to be similar to that in internodal cells (4 kg m^{-3}; KAMIYA and KURODA 1958), we obtain the gravity force per unit surface acting on the endoplasmic layer $f_g = 2 \cdot 10^{-4}$ N m^{-2} (= 0.002 dyne cm^{-2}). Assuming a linear relation between force and velocity, we can evaluate the motive force for normal streaming in rhizoids as being about 30 times the gravity force, thus about $6 \cdot 10^{-3}$ N m^{-2} (= 0.06 dyne cm^{-2}). This is about 25 times less than the mean motive force measured in internodal cells of *Nitella* (KAMIYA and KURODA 1958, TAZAWA 1968) and about 60 times less taking the values estimated by DONALDSON (1972) as a basis. As a maximal motive force for the green *Nitella* cells a much higher value has been estimated (ALLEN 1976). This indicates that the motive force per unit area in rhizoids is considerably lower than in internodal cells. The difference between the endogenous motive forces for acropetal and basipetal streaming, being on average 5-fold the gravity force, would amount to about $1.0 \cdot 10^{-3}$ N m^{-2} (= 0.01 dyne cm^{-2}). In the normal vertical position of the rhizoid, this difference value amounts to $1.4 \cdot 10^{-3}$ N m^{-2} (= 0.014 dyne cm^{-2}).

It should, however, be remembered that the difference between the endogenous motive forces for acropetal and basipetal streaming varies in different rhizoids. The highest observed difference, being ⅓ of the mean motive force, was estimated as about $2 \cdot 10^{-3}$ N m^{-2} (= 0.02 dyne cm^{-2}).

In contrast to the high variability of streaming velocities due to endogenous forces, the measured effect of the gravity force is relatively uniform and shows little variation. In the case of cytoplasmic streaming, therefore, the perception of gravity is not related to a special sensory system as is demanded for the gravitropic bending of the rhizoid (SIEVERS and VOLKMANN 1979) and for graviresponses in higher plants (VOLKMANN and SIEVERS 1979). In the streaming cytoplasm of *Chara* cells, the flowing endoplasm mass per se is effected by gravity.

4.2. Differences in Acropetal and Basipetal Streaming Velocities and the Effect of CB

After application of CB, the acropetal and basipetal streaming velocities were reduced. The difference between them, however, was constant. The basipetal streaming stopped earlier than the acropetal streaming. These facts allow some conclusions concerning the microfilament system underlying the endoplasmic streaming. To interprete the observed phenomena, we take into account that (i) the component which is essential for generating the motive force is located in the interface between moving endoplasm and stationary ectoplasm (NAGAI and REBHUN 1966, NAGAI and KAMIYA 1977, KAMITSUBO 1980) and is inhibited by CB (NAGAI and KAMIYA 1977); and (ii) *in vitro*, CB is bound specifically to the "barbed" end of the actin filament (MACLEAN-FLETCHER and POLLARD 1980, BROWN and SPUDICH 1981, POLLARD and CRAIG 1982). Thus, the effect of CB depends on the number of filament ends. In a rhizoid, during the same period of CB action, the decrease of velocity is similar in both streaming bands. This means that the magnitude of the difference between the velocities is continued. Therefore, we conclude that the two parts of the actin system underlying the opposite streams do not differ in the number of their filament ends. Otherwise, the effect of CB would be stronger on one part of the streaming and the difference between the opposite streaming velocities would not be constant. Yet, the latter was always the case in the untreated as well as in the CB treated rhizoids.

Since the basipetal streaming stopped earlier than the acropetal streaming, the microfilaments underlying this part could be shorter than that underlying the acropetal streaming. While the shorter filaments were already completely obstructed by the drug, the longer filaments could still function in a reduced way. However, it cannot be excluded that other factors such as the network of the filaments itself also play a role in the differential reaction of the streaming endoplasm after treatment with CB.

An earlier stop of the basipetal streaming was not observed in the case of inhibition with NEM. This reagent acts on the system generating motive force from the side of myosin (CHEN and KAMIYA 1975, KAMIYA 1981); it therefore functions in a manner obviously different from CB. Also, in the case of the non-controlled transient stopping of streaming which was occasionally observed, both streams stopped simultaneously.

It thus seems that the perpetuation of a constant component in the difference between acropetal and basipetal velocities during CB action is specifically related to the mechanism of CB action on actin filaments. The most plausible mode of CB action on streaming would be via the ends of the actin filaments similar to the CB action on filament elongation.

Thus, even without the knowledge of how the system generating the motive force for streaming is constructed in *Chara* rhizoids, these studies give useful hints for further detailed research on the structures involved.

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References

ALLEN, N. S., 1976: Undulating filaments in *Nitella* endoplasm and motive force generation. In: Cell Motility (GOLDMAN, R., POLLARD, T., ROSENBAUM, J., eds.), pp. 613—621. Cold Spring Harbor Laboratory.

— 1980: Cytoplasmic streaming and transport in the Characean alga *Nitella*. Can. J. Bot. **58**, 786—796.

— 1981: Motility. J. Cell Biol. **91**, 148 s—155 s.

BOTTELIER, H. P., 1934: Über den Einfluß äußerer Faktoren auf die Protoplasmaströmung in der *Avena*-Koleoptile. Amsterdam: de Bussy.

BRECKHEIMER-BEYRICH, H., 1949: Über die Wirkung zentrifugaler Kräfte auf das Protoplasma von *Nitella flexilis*. Ber. dtsch. bot. Ges. **62**, 55—60.

BROWN, S. S., SPUDICH, J. A., 1981: Mechanism of action of cytochalasin: evidence that it binds to actin filament ends. J. Cell Biol. **88**, 487—491.

CHEN, J. C. W., 1973: Observations of protoplasmic behavior and motile protoplasmic fibrils in cytochalasin B treated *Nitella* rhizoid. Protoplasma **77**, 427—435.

— KAMIYA, N., 1975: Localization of myosin in the internodal cell of *Nitella* as suggested by differential treatment with N-ethylmaleimide. Cell, Structure and Function **1**, 1—9.

DONALDSON, J. G., 1972: The estimation of the motive force for protoplasmic streaming in *Nitella*. Protoplasma **74**, 329—344.

EWART, A. J., 1903: On the physics and physiology of protoplasmic streaming in plants. Oxford: Clarendon.

FORSBERG, C., 1965: Nutritional studies of *Chara* in axenic cultures. Physiol. Plant. **18**, 275—290.

HAYASHI, T., 1957: Some aspects of behavior of the protoplasmic streaming in plant cells. Bot. Mag. (Tokyo) **70**, 168—174.

HEJNOWICZ, Z., SIEVERS, A., 1981: Regulation of the position of statoliths in *Chara* rhizoids. Protoplasma **108**, 117—137.

KAMITSUBO, E., 1980: Cytoplasmic streaming in Characeen cells: role of subcortical fibrils. Can. J. Bot. **58**, 760—765.

KAMIYA, N., 1950: The rate of the protoplasmic flow in the myxomycete plasmodium. I. Cytologia **15**, 183—193.

— 1959: Protoplasmic streaming. Protoplasmatologia VIII/3 a. Wien: Springer.

— 1981: Physical and chemical basis of cytoplasmic streaming. Ann. Rev. Plant Physiol. **32**, 205—236.

— KURODA, K., 1958: Measurement of the motive force of the protoplasmic rotation in *Nitella*. Protoplasma **50**, 144—148.

KERSEY, Y. M., HEPLER, P. K., PALEVITZ, B. A., WESSELS, N. K., 1976: Polarity of actin filaments in Characean algae. Proc. Nat. Acad. Sci. U.S.A. **73**, 165—167.

KESSLER, J. O., 1979: Gravity sensing, polar transport and cytoplasmic streaming in plant cells. The Physiologist, Suppl. **22**, S-47-S-48.

KOOP, H.-U., KIERMAYER, O., 1980: Protoplasmic streaming in the giant unicellular green alga *Acetabularia mediterranea*. II. Differential sensitivity of movement systems to substances acting on microfilaments and microtubuli. Protoplasma **102**, 295—306.

MACLEAN-FLETCHER, S., POLLARD, T. D., 1980: Mechanism of action of cytochalasin B on actin. Cell **20**, 329—341.

MUSTACICH, R. V., WARE, B. R., 1976: A study of protoplasmic streaming in *Nitella* by laser Doppler spectroscopy. Biophysic. J. **16**, 373—388.

NAGAI, R., HAYAMA, T., 1979: Ultrastructure of the endoplasmic factor responsible for cytoplasmic streaming in *Chara* internodal cells. J. Cell Sci. **36**, 121—136.

— KAMIYA, N., 1977: Differential treatment of *Chara* cells with cytochalasin B with special reference to its effect on cytoplasmic streaming. Exp. Cell Res. **108**, 231—237.

NAGAI, R., REBHUN, L. J., 1966: Cytoplasmic microfilaments in streaming *Nitella* cells. J. Ultrastruct. Res. **14**, 571—589.

NOTHNAGEL, E. A., BARAK, L. S., SANGER, J. W., WEBB, W. W., 1981: Fluorescence studies on modes of cytochalasin B and phallotoxin action on cytoplasmic streaming in *Chara*. J. Cell Biol. **88**, 364—372.

— WEBB, W. W., 1982: Hydrodynamic models of viscous coupling between motile myosin and endoplasm in Characean algae. J. Cell Biol. **94**, 444—454.

POLLARD, T. D., CRAIG, S. W., 1982: Mechanisms of actin polymerization. Trends in Biochem. Sci. **7**, 55—58.

SATTELLE, D. B., BUCHAN, P. B., 1976: Cytoplasmic streaming in *Chara corallina* studied by laser light scattering. J. Cell Sci. **22**, 633—643.

SEAGULL, R. W., HEATH, J. B., 1980: The differential effects of cytochalasin B on microfilament populations and cytoplasmic streaming. Protoplasma **103**, 231—240.

SIEVERS, A., VOLKMANN, D., 1979: Gravitropism in single cells. In: Encyclopedia of Plant Physiology, N.S., Vol. 7: Physiology of Movements (HAUPT, W., FEINLEIB, M. E., eds.), pp. 567—572. Berlin-Heidelberg-New York: Springer.

TAZAWA, M., 1968: Motive force of the cytoplasmic streaming in *Nitella*. Protoplasma **65**, 207—222.

VOLKMANN, D., SIEVERS, A., 1979: Graviperception in multicellular organs. In: Encyclopedia of Plant Physiology, N.S., Vol. 7: Physiology of Movements (HAUPT, W., FEINLEIB, M. E., eds.), pp. 573—600. Berlin-Heidelberg-New York: Springer.

WILLIAMSON, R. E., 1975: Cytoplasmic streaming in *Chara:* a cell model activated by ATP and inhibited by cytochalasin B. J. Cell Sci. **17**, 655—668.

— 1979: Filaments associated with the endoplasmic reticulum in the streaming cytoplasm of *Chara corallina*. Europ. J. Cell Biol. **20**, 177—183.

— 1980: Actin in motile and other processes in plant cells. Can. J. Bot. **58**, 766—772.

YAMAGUCHI, M., NAKAMURA, T., SEKINE, T., 1973: Studies on the fast reacting sulfhydryl group of skeletal myosin A: Conversion to smooth muscle myosin type with N-ethylmaleimide treatment. Biochim. biophys. Acta **328**, 154—165.