Ann. Rev. Biophys. Bioeng. 1978. 7:497–526

CYTOPLASMIC STREAMING IN GREEN PLANTS

♦9121

Nina Strömgren Allen and Robert Day Allen
Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755

INTRODUCTION

Cytoplasmic streaming is such an obvious and aesthetic feature of many of the cells of green plants that it should be no surprise that it was seen and seriously studied long before similar processes were discovered in animals, fungi, and protists.

Corti (29) is credited with the discovery of rotational cytoplasmic streaming in the giant cells of characeans. To this day more is known about streaming in *Nitella, Chara,* and other characeans than about any other example of cytoplasmic streaming in plants.

The literature on cytoplasmic streaming is rich in excellent reviews, most of which are written from a purely botanical perspective (42, 82, 83, 99–101, 107, 147, 148, 181). The lessening tendency of biologists to limit interest to either plants or animals has led to important recent reviews in which certain aspects of plant and animal cellular structure and motility have been considered together (59, 134).

The present review, like the preceding one in this journal (13), is intended to be more restricted in scope and tries to integrate the cellular and biophysical aspects of cytoplasmic streaming with the emerging biochemical information, not only in plants but in muscle and other motile systems. For background information in the study of motility, the following reviews are recommended (62, 134, 151, 175). Several recent symposia have considered many forms of motility (49, 67, 135).

Streaming is the continuous, irreversible deformation of cytoplasm at the expense of endogenous cellular energy reserves. Unlike the streaming in amoebae, the cytoplast as a whole need not change in external shape. The cytoplasm of plant cells is generally confined by rigid cell walls, but the interior areas of cytoplasm may shift. As is pointed out further on, similar non-amoeboid cytoplasmic streaming occurs in many protists, such as the ciliates *Paramecium* (102) and *Stentor,* the heliozoan Echinosphaerium (40, 41), and the foraminifer *Allogromia* (10, 70, 112).

0084–6589/78/0615–0497$1.00

However, the literature on these organisms is considered only in comparison to plants and is not reviewed in detail.

Some plant cells lend themselves admirably to experimental approaches. The giant internodal cells of characeans, for example, can be ligated (38, 39, 106), devacuolated (55), centrifuged, and subjected to various chemical treatments through the plasma membrane and/or tonoplast; their endoplasm can be removed by a simple "cell operation" (86), or endoplasmic droplets of a variety of sizes can be prepared by "stripping" the cell contents into its own vacuolar fluid (37, 74, 182). Streaming can be stopped at will by either electrical or mechanical stimulation (83). Although characean cells are naturally quite transparent, their endoplasm can be observed more clearly if a "window" is made through the layer of cortical chloroplasts by brief exposure to strong light (79). These and other important techniques have been developed largely in the laboratory of N. Kamiya, who has been the leading scientist in the field of plant streaming since the 1950s. The purpose of this review is to present a summary of what is currently understood about the mechanisms of streaming in green plants, and to point out specific problems that require further research.

The long-harbored suspicion that contractile proteins might be responsible for developing the motive force for streaming received partial confirmation when Condeelis (27) first demonstrated actin in a higher plant, *Amaryllis*. Before that, actin had been identified in the algae *Nitella* (126) and *Chara* (178), and many examples of the occurrence of microfilaments had been cited in plant cytoplasm without chemical evidence to support their identity as actin (59).

Myosin has now been identified and characterized by Kato & Tonamura (91a) from *Nitella flexilis*. In general, the state of knowledge regarding the biochemistry of motility in plant cells is considerably less advanced than in animal and protistan cells. Some of the reasons for this are discussed.

The mechanisms of streaming in plants continue to be unclear. Kamiya's active shearing theory (85), which invoked an unspecified "active shearing" force at the border between the cortex and endoplasm, requires revision in the light of the discovery (3) that particles saltate along filaments not only in the subcortical layer but throughout the endoplasm. The nature of the saltatory interaction seems best approximated by the general model of Schmitt (146), which may apply broadly to cytoplasmic transport processes. The discovery that endoplasmic filaments undulate suggests either that the undulations assist in propelling the fluid or that undulations might be a passive byproduct of the saltatory interaction. The question of whether the ground cytoplasm, the particles within it, or both are actively driven in cytoplasmic streaming has been with us a long time, judging from reviews (82, 83, 138). It is still unsolved, but at least now it appears to be phrased in a way that experiments can test.

PHENOMENOLOGY

Kamiya's excellent reviews (82, 83) delineated five basic types of streaming in cells of green plants: (*a*) agitation, here renamed saltation; (*b*) circulation; (*c*)

rotation (cyclosis); (*d*) fountain or reverse-fountain; and (*e*) striatial (or multistriate) streaming. Among fungi, two additional types are found: shuttle streaming in slime molds and sleevelike streaming in fungal hyphae. These patterns of streaming are not always distinct from one another as variants and mixtures of types are seen as well as developmental progressions from one streaming type to another as cells differentiate (82). These same patterns are not restricted to plant cells, but are found in various protistan, animal, and fungal cells as well. It is also necessary to mention quite a few "miscellaneous examples" of motility in plant cells that may be related to streaming, including phloem transport.

Saltation

The least organized and perhaps the simplest and most widespread form of streaming is the independent motion of particles that was originally called *Glitchbewegung* (120) and then *agitation* (82, 83) and is now generally referred to as *saltation.*

Saltation has been carefully studied in a number of cell types (130, 136–140, 156) and has been characterized as discontinuous rectilinear or curvalinear excursions (or "jumps") of cytoplasmic particles that exceed the distance expected to be moved by such a particle because of Brownian motion. From the size of a particle, Einstein's equation [$\bar{x}^2 = (kT/3\pi\eta a)t$, where k is Bolzman's constant, T is absolute temperature, η is viscosity in poise, a is particle radius, and t is total time of observation (139, 148)] predicts the mean square displacement of that particle in a fluid of known or assumed viscosity. For example, Rebhun (138) calculated that over an interval of 10 sec, particles 1.0 μm in diameter would show a mean square displacement of 0.09 μm in a fluid with a viscosity of 10 P, and 2.3 μm in a fluid with viscosity of 0.01 P. Since the viscosity of cytoplasm would always be greater than that of water, the expected mean square displacement of a given particle in water would appear to approach the upper limit for expected Brownian excursions. A particle motion well in excess of that limit is clearly a saltation. It is much more difficult to decide whether or not excursions over marginal distances are saltations because the rheological properties of cytoplasm are not fully understood. The view that cytoplasm is a Newtonian fluid of low viscosity (58) was shown to be incorrect in amoebae by Allen (8, 9), who demonstrated that centrifugal accelerations used to sediment particles in cells exerts shear stresses in excess of the yield point of cytoplasm and thus destroy the cytoplasmic structure while probing its properties. Hiramoto has confirmed and extended these results on sea urchin egg cytoplasm (61). The Einstein equation is valid only for Newtonian fluids, and the assumption that cytoplasm is Newtonian is at best a shaky one. Allen (5) measured the mean square displacement of particles in quiescent cytoplasm of stimulated *Nitella* internodal cells and calculated a mean viscosity of roughly 0.06 P. Since Kamiya & Kuroda (88, 91) measured a much higher apparent viscosity by determining the force required to aspirate an endoplasmic droplet of *Nitella* into an agar capillary, one might suggest that *Nitella* cytoplasm in bulk has a relatively high apparent macroviscosity due to the presence of endoplasmic filaments, endoplasmic reticulum, and other structures, and a relatively low microviscosity of the fluid in the interstices (5). Very long saltations (>100

μm) occur regularly along these endoplasmic filaments, but only Brownian motion of tiny particles occurs between them.

Jarosch (71) described a simple test for the regularity of particle motion. In Brownian motion the mean square displacement of a particle is proportional to the interval over which its displacement is measured. On the other hand, for unidirectional motion, the mean square displacement is the square of the interval. Consequently, if one compares the mean square displacement at one interval, *i*, and then at double that interval, the regularity in direction of the movement will be expressed in the regularity coefficient, or the ratio of the greater to the lesser displacement. The coefficient will be 2 for perfectly random motion (Brownian motion) and 4 for unidirectional motion, such as that in streaming. Jarosch (71) used this simple test to document quantitatively the gradual increase in the directionality of particle motion as a function of time in epidermal cells of *Allium.*

The tendency of saltations to follow straight or gently curving paths suggest that saltating particles associate in some manner with linear elements, such as microfilaments, microtubules, 100-Å filaments, or perhaps some form of thick filament. The most likely candidate for the motive force system for saltation is considered to be actin- and myosin-like proteins, in the view of Rebhun (138) whose excellent review summarizes the evidence up to 1972. The case of saltation most studied recently in plants is the transport of particles along subcortical fibrils (79, 81) and endoplasmic filaments (5) in *Nitella.* The subcortical fibrils, of which the endoplasmic filaments are branches (11), are bundles of polarized F-actin filaments (126). Therefore, the model of Schmitt (146), independently proposed by Williamson (180) and Kamiya (84), comes to mind as a plausible explanation of the interaction of a particle, to which myosin is attached, with a bundle of F-actin filaments. Huxley (65) has suggested an interaction of soluble myosin molecules with F-actin filaments, and Tirosh et al (168) have described "streaming" in actomyosin solutions.

One cannot exclude the possibility that microtubules (or 100-Å filaments) might constitute part of the mechanism of saltation in some instances, because some very active saltation takes place near spindles, within centrosomes, and adjacent to microtubular organelles, such as in the axoneme of *Echinosphaerium* (167). Although the elegant experiments of Edds (40, 41) established that the microtubules do not provide the motive force for saltation in *Echinosphaerium,* in that cell actin is present, along with as yet unidentified "thicker" filaments. It is not certain to what extent this result can be generalized, expecially in plant cells.

Circulation

Circulation is seen in the cortex of many plant cells and in the transvacuolar strands. The pattern of circulation is usually stable over the short term (minutes), but the transvacuolar strands themselves gradually alter their location, splitting and joining strands, as do streams in the cortical region. Circulating particles may move unidirectionally or bidirectionally at velocities that may vary from barely perceptible motion to 40 μm sec^{-1}. Excellent examples of circulation can be found in the hair cells of *Tradescantia, Urtica* (stinging nettle hair), *Gloxinia,*

Cucurbita, Campanula, and *Saxifraga,* and in parenchymal cells of *Allium* and other monocotyledonous plants. The most recent study of circulation was that of T. Salmon and B. Burnside (unpublished film), who demonstrated the interaction between cytoplasmic particles and fibrillar elements in the stamen hair cells of the tropical plant *Setcreasea purpurea,* a relative of *Tradescantia.* In electron micrographs, these structures resembled bundles of F-actin, but heavy meromyosin (HMM) labeling was not performed. *Euphorbia* tissue culture cells show a circulatory streaming pattern with typically anastemosing transvacuolar strands with movement (sometimes bidirectional) of both cytoplasm and organelles at velocities from 0–19 μm sec^{-1} (109). One important observation Mahlberg (109) made was that streaming velocity in a thick transvacuolar strand at its widest diameter did not change when it reached a constricted part of the strand. He noted that the constricted portion contained fewer "microstreams" and that these seemed to connect to the thicker part of the strand. Mahlberg's microstreams are probably very similar to the fibrils observed by Salmon and Burnside.

Caulpera has definite bands ca 100 μm wide in which files of chloroplasts stream bidirectionally at 3–5 μm sec^{-1} in a circadian rhythm. This form can also be classified with multistriate streaming. The bands correspond to bundles of microtubules, and on the strength of their localization, it was suggested that they might play a role in this example of circulation (143). No microfilaments were seen, and no colchicine experiments were reported. Since at the time of that study microfilaments in nonmuscle cells were frequently not well preserved by fixation (153), it would be advisable to reexamine the ultrastructure of *Caulerpa* to look not only for evidence of microfilaments, but also for signs of microtubule-microfilament interaction. *Caulerpa* also may be a suitable material in which to examine some of the biochemical aspects of cytoplasmic circulation.

Fountain, Reverse-Fountain Streaming

Fountain and reverse-fountain streaming (16) are more ordered than saltation and circulation, but less ordered than rotation. As Kamiya (83) pointed out, reverse-fountain streaming is far more common than fountain streaming, and it often shows a developmental transition toward cytoplasmic rotation. This is especially evident in some pollen tubes (69). True fountain streaming is found in pollen tubes of *Plantago* and *Lillium* (97), and reverse-fountain streaming is found in root hairs of *Tradescantia* and in pollen tubes of many other plants, including *Amaryllis,* in which Condeelis first demonstrated actin in a higher plant cell by HMM binding to microfilaments of isolated cytoplasm.

Multistriate Streaming

Acetabularia, a marine coenocytic green alga 2–10 cm long, and *Phycomyces,* a fungus about 5 cm long, have very similar patterns of streaming and cellular organization. They have a large central vacuole surrounded by a tonoplast, a layer of motile endoplasm, a rigid cortex, and a cell wall. Streaming occurs along tracks or channels with quiescent cytoplasm separating these channels. Some channels are very close to the ridges on the cortex (83).

In *Acetabularia,* chloroplasts, mitochondria, and other particles move at rates from 1–10 μm sec^{-1} in both directions. Particles will jump from one stream to another occasionally. One track normally has streaming in a single direction, but reversal occurs rarely, either naturally or due to a change in the quality of light (176). Red light (>640 nm) causes a slow decrease in streaming velocity and an apparent interaction between strands. If cells are kept in red light, a brief exposure to low-intensity blue light (480 nm) following red light doubles the streaming velocity after a delay of 1–3 min and the number of strands increases (176). There may be as many as three types of strands: one in which streaming is slow and particles are situated close to cortical ridges; one in which particles appear to be enclosed in an envelope; and single thin strands along which particles move.

Rotational Streaming

Rotational streaming is the most organized and efficient form of streaming in green plants and as a rule is restricted to a belt of cytoplasm that streams around the cell periphery. In leaves such as those of *Elodea,* the cytoplasm streams almost entirely over the cell surfaces that are in contact with other cells (83). Under constant conditions of light, temperature, and environmental chemistry, the rate of streaming is remarkably constant day and night. However, seasonal variations in rate are common.

Although many different fluids of plant cells exhibit rotational streaming, we concentrate on *Nitella* and other characean cells, because they are the materials that have been selected for most of the experimental studies on streaming in plants.

Nitella plants are found in relatively shallow water in lakes and ponds of the temperate zone. Their colorless rhizoid cells lack differentiated chloroplasts and are especially suitable for optical studies. However, they are delicate, do not survive well in the laboratory, and are unsuitable for many experiments. Internodal cells are from 1–5 cm long and can be kept isolated from the whole plant for weeks. Leaf cells are somewhat smaller and less transparent.

A typical internodal cell might be 2–5 cm long and 1 mm in diameter. A band of moving cytoplasm that is semicircular in cross-section, the endoplasm, streams spirally along the cell in one direction, turns around at the end of the cell, and streams back, occupying the opposite half of the cross-section of the cell. Separating the two oppositely directed streams are two spiral "indifferent zones" where the endoplasm is either lacking or very thin.

Pickard (131) studied the "ends" of cells where the cytoplasm rounds a curve and concluded that they were similar in nature to the rest of the endoplasm. The endoplasm is sandwiched between the stationary cell cortex on the outside and the tonoplast, which separates the endoplasm from the large central vacuole. The cortical layer contains evenly spaced spiral rows of stationary chloroplasts. Additional free chloroplasts may be seen in the endoplasm, where they are often rotating on one of their axes. *Nitella* cells are multinucleate, and some nuclei also rotate.

Kamiya & Kuroda (85) measured streaming velocities at different depths within the endoplasm and vacuole and constructed a velocity profile in which it could

be seen that the bulk of the endoplasm streamed at an almost uniform velocity. In some characean cells, this velocity may approach 100 μm sec^{-1}. Since the maximum velocity is reached by particles less than 2 μm from the cortex, it appeared that the mean velocity gradient between the moving belt of endoplasm and the stationary belt of cortical cytoplasm is ca 50–100 sec^{-1}. The degree of uniformity of particle velocities in the endoplasm is in some doubt. The data of Kamiya & Kuroda (85) show variations within the limits of experimental error. However, Kamiya (83) published a photokymograph recording of streaming in a *Chara* rhizoid that showed traces made by endoplasmic particles. The traces are not all parallel, which suggests less uniformity in particle velocity than does the text. These experiments were carried out before optical systems with shallow (and known) depths of field were available. Preliminary analysis of films indicates that small variations do indeed exist in the velocity in neighboring regions of the endoplasm (N. S. Allen, unpublished data). These local variations may be important and should be studied under rigorously controlled optical conditions.

The apparent plug-flow velocity profile with its steep velocity gradient adjacent to the cortex led to the active shearing theory (85), which was in accord with the earlier concepts of Linsbauer (106) and of Breckheimer-Beyrich (22) that localized the motive force at or immediately adjacent to the inner surface of the cortical layer.

The interpretation of Kamiya & Kuroda's (85) velocity profiles was that shear (a velocity gradient) was developed only at the inner border of the cortex. The subsequent discovery of endoplasmic filaments anchored as branches of the subcortical fibrils (5) invalidated this interpretation, for shear occurs not only at the inner border of the cortex, but also at the surface of every endoplasmic filament.

Therefore, structures that could produce the motive force for streaming were looked for in accordance with ideas prevalent at the time at the border between the cortex and endoplasm. In 1966, Kamitsubo (78) demonstrated photomicrographically a population of subcortical fibrils (a term we prefer over "cables," which implies twisted elements, and "fibers," which are usually large enough to see easily) that run along the inner surfaces of the rows of chloroplasts. Particles have been recorded saltating in contact with the subcortical fibrils at velocities higher than, similar to, and lower than endoplasmic streaming velocity (6, 79, 116).

Using Kamitsubo's (80) technique for removing the chloroplasts in a ~100 μm-diameter "window" in the surface of a *Nitella* internodal cell, Allen (5) observed and recorded streaming movements under exceptionally good optical conditions by differential interference microscopy using laser illumination. The endoplasm was shown to contain a large number of undulating filaments estimated to be 0.1 μm or less in diameter and long in comparison to the field diameter of the microscope. Counts of the number of filaments per optical section were used to calculate that the total length of filaments in a single cell 2 cm long was over 50 M. The observed undulations varied in amplitude and wavelength, but a typical filament showed a sinusoidal wave with a wavelength of 25 μm and an amplitude of 5 μm. With hydrodynamic formulas developed for estimating the propulsive

force and predicted propulsion velocity of sperm, Allen (5) was able to predict the streaming velocity within a factor of 3, and to show that the motive force developed by the total complement of undulating filaments was more than sufficient to drive the fluid cytoplasm around the cell.

Under the best of conditions, the filaments were barely visible because of their size. However, they could be seen in stroboscopic illumination by the behavior of spherosomes that saltate along them in sinusoidal arrays and serve as marker particles. In stroboscopic illumination it can be seen that nearly all of the larger cytoplasmic particles (spherosomes) are attached to endoplasmic filaments.

When internodal cells are stimulated either mechanically or electrically, streaming stops for up to a minute and then gradually regains its original velocity. When streaming in the cytoplasm was stopped, straight filaments could be seen throughout the endoplasm. They varied in size and in contrast, which suggests that the thinner filaments were considerably below the limit of resolution (0.2 μm). The first motions to be observed were saltations along both the subcortical fibrils and endoplasmic filaments simultaneously. As the velocity increased, the filaments became more difficult to see because of their undulation.

As far as could be determined light microscopically, endoplasmic filaments are branches of the subcortical fibrils that hang downstream, undulate, and either transport particles or serve as tracts for the independent motion of particles (5). Many branch points could be seen in light microscope films (4) and in scanning electron micrographs (6, 7) in all endoplasmic layers. Subcortical fibrils can be seen to branch (6, 7, 80).

These observations on the endoplasm have led to the suggestion that two possibly related mechanisms may be operating in rotational streaming.

1. Saltation of particles occurs along subcortical fibrils and endoplasmic filaments. The drag forces caused by the movement of particles in the endoplasm might then cause the fluid to flow first by internal shear around the particles and then by plug flow.

2. Undulation of filaments could be responsible for the bulk flow of the endoplasm in addition to the forces delivered by saltation. Considering that most streaming cells have saltations that are between 1–10 μm sec $^{-1}$, and in *Nitella* rotational streaming occurs from 50–100 μm sec $^{-1}$, it is possible that the undulations produce force over and above that of saltations.

Possibly Related Forms of Plant Motility

A complete catalog of plant motility (which this review is not!) would include many diverse forms of motility that have hardly been studied.

Cellular migration occurs into or across intracellular bridges that lead to syngamy in conjugates such as *Spirogyra.* In *Acetabularia,* the single nucleus found in a rhizoid gives rise to about 1000 much smaller nuclei, which migrate to the cap, where cysts are formed. This unidirectional movement occurs in cytoplasm that streams bidirectionally (53). Chloroplasts of may cells are rotated, shifted, or translated in response to changes in the quality or quantity of light that reaches the

cell. Such motions, caused by light, are referred to as *photodinesis* and occur, for example, in *Chromulina, Ulva, Hormidium, Ulothrix, Bryopsis, Mougeotia, Mesotaenium,* and *Vaucheria* (121), and also in *Vallisneria, Elodea, Lemna,* and *Funaria* (183). Some of these movements in *Mougeotia* and *Mesotaenium* are phytochrome mediated (54). Other examples of photodinesis are blue light responses mediated by carotenoids or flavines (183).

In *Mougeotia,* changing from low to high light intensities leads to immediate increased saltations and the formation of filaments that connect the cortex to the chloroplast (12). The chloroplast subsequently rotates 90° on its long axis over a period of 15–20 min. These responses are inhibited by cytochalasin B (171). Marchant (110) has shown HMM binding in the filaments in extracts of *Mougeotia* and *Coleochaete* cells. Unfortunately, we do not know the localization or polarity of these filaments.

Is Transport in Phloem Related to Streaming?

We would be remiss if we did not include mention of a long-standing controversy regarding the possible relationships between the mechanisms of phloem transport and streaming that has existed since DeVries (34) suggested that protoplasmic streaming in phloem served to transport nutrients. Several workers have perceived this possible relationship, among them Biddulph (17) and Swanson & Böhning (152). On the basis of ultrastructural data, Thaine (163) proposed the transcellular protoplasmic theory of translocation based on the finding that transcellular strands interconnect sieve cells through sieve pores. Others who have supported this notion are Johnson & Weatherley (76, 173), Thaine (164), Aikman & Anderson (2, 14), Lee et al (105), MacRobbie (108), Fensom & Williams (43, 44), and Robidoux et al (141).

Reviews present the arguments for and against the involvement of streaming or some other form of motility (25, 31, 59, 172). Phloem is a difficult tissue on which to make in vivo observations because surrounding tissue must be dissected away without interfering with the phloem.

Cronshaw & Esau (32) discovered "P-protein" filaments that normally occupy the lumen of the sieve elements, and in healthy cells traverse from one element to the next through the sieve pores. Fixatives have a profound yet variable effect on the presence and location of the P-protein. It may "contract" to form slime bodies that plug the sieve pores (114). Perhaps the best preservation has been achieved by freeze-substitution on celery petioles (33).

When phloem exudates from 10 different plant species were fractionated and compared by polyacrylamide gel electrophoresis, the major exudate proteins were found to vary greatly among species and no component co-migrated with actin (149). On the basis of present evidence it would appear that phloem exudate proteins are a very heterogeneous and variable group. It is quite possible that the ultrastructurally defined P-protein does not appear in the exudate, and the F-actin filaments could be present in the sieve elements and not appear in the exudate.

Cytochalasin B, which inhibits cytoplasmic streaming in some plants but not in amoebae, inhibits sucrose transport in *Heracleum* (165), but not in *Lepidium* (177).

P-protein is unaffected by colchicine but is precipitated by vinblastine sulfate and calcium (174).

There has been some speculation on whether P-protein might be actinlike or contractile (43, 108). Neither Williamson (177) in *Ricinus* nor Palevitz (124) in *Phaseolus* found HMM labeling in P-protein. However, Ilker & Currier (66) report HMM binding to P-protein in *Vicia* and *Xylosma,* but the micrographs are not convincing. It is suggestive that a myosinlike Mg^{2+} ATPase was found in phloem cells (47); however, before being implicated in motility, its actin activation should be tested.

At the present time there is insufficient evidence to settle the controversy as to whether or not streaming and phloem transport are related. Even transport rates in Phloem are controversial. Because of the small amounts of phloem available for biochemical work, this is a technically challenging problem.

ULTRASTRUCTURE

Evidence from Light Microscopy

There is no doubt that the subcortical fibrils, endoplasmic filaments, and other motile filaments seen in the endoplasmic droplets discussed later are detectable because they possess a higher refractive index than their surrounding cytoplasm. It can also be seen in a high extinction polarizing microscope that they show weak positive axial birefringence (N. S. Allen, personal observation).

Scanning Electron Microscopy

Kersey & Wessells (95) fixed whole cells of *Chara australis* and cut them in half longitudinally. Thus the chloroplast rows were seen from the inside with as many as six intact subcortical fibrils extending over one file of chloroplasts and no indication whatever of endoplasmic filaments or other structures. However, there are some particles in close association with the fibrils.

Allen, Allen & Reinhart (7, 11; N. Allen, R. Allen, and T. Reinhart, manuscript in preparation) have prepared *Nitella* sp. cells in a somewhat different way and thus preserved much more of the structure visible in intact cells with the light microscope. Some preparations cut open and viewed from the inside show the tonoplast largely intact, but torn in places to reveal a vast array of partially oriented endoplasmic filaments from 80–410 nm in diameter and of indefinite length (11). In other preparations these filaments are somewhat less well oriented, and in many areas the filaments have been "blown away" to varying degrees to reveal the chloroplast rows, subcortical fibrils, and small broken branches where the endoplasmic filaments originally emerged from them (11).

Thus there seems no doubt concerning the reality of the endoplasmic filaments, particularly since one of their characteristic features is often (but not always) well preserved: their associated sphaerosomes. The diameters of these filaments

explain why their light microscope images were so faint, especially while they were in motion. Although it is well known that structures below the resolution limit of the light microscope can be seen if they exhibit enough contrast, the size they appear in the image is never smaller than an airy disk. However, the image becomes less contrasty as the object size decreases (5). It is suspected that some of the smaller filaments were not detected microscopically, which suggests that the estimated 50 m of filaments per cell (5) may be a minimum figure.

Transmission Electron Microscopy

Of the many ultrastructural studies of plant cells, few relate specifically to motility. O'Brien & Thimann (122) were the first to observe 50- to 80-Å microfilaments in cells of a higher plant (Avena coleoptile). Parthasarathy & Mühlethaler (129) showed microfilament bundles parallel to the long axis of elongating cells of stems and roots. The bundles were up to 12 μm long and 0.1–0.3 μm in diameter. In immature sieve elements of *Prestoea montana* they showed P-protein filaments in association with microfilaments that were bent as if undulating and associated with particles. A useful summary in tabular form of the findings of microfilaments in plant cells is given by Parthasarathy & Mühlethaler (129).

Bundles of microfilaments were also seen in the region that corresponds to the location of the subcortical fibrils in *Nitella* (119) and in *Chara* (132). It was also found that although microtubules were present, they were located in the cortical layer adjacent to the cell wall. They were not as highly oriented as in higher plants (133), and not in a position to be part of a motive force generation system. In characean cells, the membranes of chloroplasts are attached to the subcortical fibrils, as evidenced by the fact that chloroplast files are frequently held together after isolation by the subcortical fibrils (89). This attachment is seen in the electron micrographs of Palevitz & Hepler (125, 128). A similar attachment, or at least close association between microfilament bundles and mitochondria, was shown by Parthasarathy & Mühlethaler (129) in a cell from willow (*Salix*).

Another ultrastructural element that might play a role in the mechanism of streaming is the 100-Å filaments. These are present in nerves that show axoplasmic transport (48, 59). L. Rebhun (personal communication) saw occasional filaments ca 100 Å in diameter in *Nitella* sections. These have never been isolated from plants and both their function and their relationship to other 100-Å filaments in animal cells is unknown.

Microfilaments from *Nitella* cytoplasmic suspensions show the characteristic double helical crossover ($i = 360$–380 Å) and bind rabbit skeletal muscle HMM in the characteristic arrowhead arrays (68) that show they are F-actin. In bundles, they are paracrystalline and polarized with all of the arrowheads pointing in the same direction (126). As discussed earlier, Kersey et al (94) showed that the arrowheads all point opposite to the direction of streaming and are released in the presence of 2 mM ATP. Williamson (178) found S1 produced arrowheads on *Chara* actin.

Kersey et al (94) pointed out that negatively stained endoplasm, freshly removed, contains few demonstrable F-actin filaments. Similarly, no good electron micro-

graphs have been published that show the endoplasm and intact tonoplast of characean cells. Fixation is more of a problem with these giant algal cells than many have realized. Preparation of fixed cells for scanning electron microscopy showed that cells fixed intact shrink and are severely deformed by fixation. Cutting the cells into short fragments immediately on addition of the fixative helps, but even so there is great variability in the quality of fixation in different fixatives and in different preparations with the same fixative. The large central vacuole of these cells is quite acidic and contains ionic concentrations quite different from the cytoplasm. Taking all of these factors into account, it is not surprising that there is still far too much uncertainty concerning the state of preservation of the cytoplasm. This situation is not unique to characean cells; the companion review (13) on cytoplasmic streaming in amoebae stresses the poor preservation of amoeba cytoplasm as a serious obstacle to the study of the mechanisms of streaming at the ultrastructural level. A problem that is very likely common to the two systems is the lability of F-actin filaments that are unprotected by tropomyosin (153). Too little is known about possible actin-binding proteins in characean cells to speculate on their role in fixation.

It is possible that many of the problems inherent in chemical fixation will be circumvented by rapid-freezing methods followed by substitution with organic solvents or by freeze-fracture.

There has been slow but significant progress in obtaining better endoplasmic fixation in *Nitella* by monitoring cell preparations by viewing samples in the scanning electron microscope (N. Allen, R. Allen, T. Reinhart, manuscript in preparation). Thick (1–2 μm) sections of specimens known to have intact endoplasmic filaments after fixation have been viewed with the 1-MeV electron microscope facility at Boulder, Colo. Longitudinal sections of these preparations show well-preserved subcortical fibrils associated with chloroplast membranes as already demonstrated by Palevitz (125). However, one also sees branches of the subcortical fibrils leaving the cortical layer in the direction of the endoplasm. They can be seen to be thinner bundles of the F-actin known to be present in the subcortical fibrils. Therefore, it is not surprising that if particles can saltate along the subcortical fibrils, they can do the same in contact with endoplasmic filaments.

The experience to date with the unreliability of negative ultrastructural results should lead us to be cautious in interpreting the apparent lack of thick filaments of any kind in characean cells. Myosin in a plant cell has just been demonstrated (91a). We now must look for the presence and location of myosin molecules in situ.

BIOCHEMISTRY OF MOTILITY IN PLANTS

Our present perspective regarding the biochemical basis of motility is somewhat distorted by the fact that the biochemistry of muscle was investigated first and supplied us both with a body of indispensable information and with many preconceptions about how the biochemistry of other forms of motility should be pursued and interpreted. Many early biologists, among them Wilhelm Pfeffer (130), pre-

dicted a basic similarity in the chemical aspects of fundamental cellular processes. The statement of Monod & Jacob (see 24a) that ". . . anything found in *Escherichia coli* must also be true of elephants" may have been too strong, for the bacterial flagellar motile system appears to be limited to procaryotes. Nevertheless, considerable interest has been aroused by the discovery of proteins in some procaryotes that have several (but far from all) properties of actin (142). If this apparent similarity of some procaryotic proteins to actin continues to survive experimental tests, it may be found that actin has functions other than its role in motility. It is already suspected that actin plays a cytoskeletal role in many cells (cf 13).

Although it is not yet established that actin is present in all eucaryotic cells, it has been demonstrated to be present in representative cells of all four kingdoms of eucaryotes. It is probably significant that actin was discovered in cells of higher plants last. Condeelis (27) isolated cytoplasm from *Amaryllis* pollen tubes and demonstrated F-actin filaments by specific HMM binding released by ATP. Forer & Jackson (45, 46) have demonstrated actin by the same criterion in *Haemanthus* endosperm. The first demonstration of actin in a green alga was in *Nitella* and *Chara*. The subcortical fibrils were labeled with HMM (126, 127, 178), showing that the HMM arrowheads all pointed in the same direction in one subcortical fibril, but the direction of streaming relative to the polarity of the actin was unknown. Kersey (93, 94) marked the direction of streaming with a dye spot, which remained in place after fixation. Consequently, she was able to show that the HMM arrowheads point in the direction opposite to streaming.

There are no published reports that detail the isolation and characterization of actin from any plant. Acetone powders from *Nitella* contain a protein that co-migrates with muscle actin (125). Actin extracted from *Nitella* with 0.6 M KI also co-migrates with muscle actin (N. S. Allen and J. S. Condeelis, unpublished data). However, *Nitella* actin prepared in this way degrades rapidly if not kept at 0°C and protected against protease digestion. Among protease inhibitors tried, trasylol proved most effective. If these precautions are taken, at least 15% of the protein of the cell appears to be actin. *Nitella* extracts also contain a 55,000-dalton component, suggestive of that in *Limulus* acrosomes (166) and in extracts of *Dictyostelium* amoebae (28). Actin, which co-migrates with rabbit actin, has been isolated from soldier beans (69a).

Nitella extracts fractionated by centrifugation showed no sign of a myosin band in the 200,000+ dalton range in the supernatant, but in the particulate fraction Mg^{2+} ATPase activity was detected, as well as a weak band over 260,000 daltons. Much remains to be done to purify this protein, if indeed it is myosin, before its enzyme activity can be assayed and its actin activation can be measured. Myosin has been extracted from 1–3 kg of *Nitella flexilis* with 0.3 M KCl, 10 mM EDTA, and 10 mM Na-PPi and 20 mM imidazole at pH 7.0 and then further purified (91a); 46,000-molecular-weight actin was also present. Like slime mold and amoeba, the molecular weight of the *Nitella* myosin is greater than 200,000 and thus greater than muscle. At low ionic strength, pH 6.5, 2-μm-long bipolar filaments were observed in the electron micrograph in negatively stained preparations. At low ionic strength, super-precipitation occured when ATP was added. At high ionic

strength, the myosin ATPase reaction was activated by EDTA or Ca^{2+} and was inhibited by Mg^{2+}. The Mg^{2+}-ATPase reaction was activated by skeletal muscle, where Ca^{2+} had no effect (91a). This means the Mg-ATPase is not Ca^{2+} sensitive.

The purification of plant contractile proteins unfortunately is complicated by the presence of polysaccharides and glycoproteins. Further problems arise because of the large, acidic vacuoles of these cells and the many proteases present. The next step is to isolate myosin from vascular plant cells, with lower streaming rates, to see if those myosins are like *Nitella* myosin.

Another approach to localization of specific proteins in cells is by antibodies used in indirect immunofluorescence (104). No reports have yet appeared using this method on plants, nor has anyone injected living plant cells with fluorescent probes specific for actin or myosin and observed their location. Fluorescent probes should be selected not to fluoresce in the same region as chloroplasts!

LASER-DOPPLER SPECTROSCOPY

A new method for the analysis of particle motions in suspension in cells or liquids is the photon correlation analysis of laser light scattered by moving particles (103, 115–117, 145). With this method it is possible to measure the distribution of velocities of particles large and small, in the endoplasm of cells such as characeans. A sharp peak measures what is described as a narrow range of velocities (±10% of mode). However, the velocity distributions do not correspond to the plug flow velocity profiles measured in the endoplasm of *Nitella* by Kamiya & Kuroda (85), but rather it is intermediate between a parabolic distribution and plug flow (116). It is not clear, for example, how one should interpret velocities on the high side of the peak velocity, as no velocities of this magnitude are seen by microscopy. It is also important to recognize that the velocity distribution measured by Doppler shifts in scattered light are not equivalent to velocity profiles, because the spatial information has been lost.

Although it is still difficult to interpret laser-Doppler velocity distributions in terms of precisely what is happening in the cell, it has been possible by this method to collect very rapidly data that would be tedious to obtain by any other method. For example, Mustacich & Ware have been able to measure accurately the photoresponse in *Nitella* (116). An important finding is that the threshold for photoinhibition of streaming increases 1000-fold when the light is administered through a "window" in the cortical layer of chloroplasts. Therefore it is the chloroplasts that have the greatest sensitivity to light, as might be expected because of their chlorophyll.

The laser-Doppler spectrum shows a category of low frequencies that probably correspond to a mixture of several phenomena, which includes diffusion and shearing in the vacuole center and adjacent to the cortex, and that possibly are in part a result of the undulation of filaments (5).

Recently, as mentioned, Mustacich & Ware (117) reported that although 1.0 mM ATP causes a 15% increase in streaming velocity in *Nitella flexilis* (134), higher concentrations increased low velocities to the Doppler spectrum. This may indicate that ATP has a "relaxing" effect on *Nitella* cytoplasm that releases sphaero-

somes from their saltatory association with subcortical fibrils and endoplasmic filaments. [See the companion review regarding the effects of ATP in relaxation solution on the cytoplasm of intact amoebae and single cell models (13).]

ISOLATED ENDOPLASMIC DROPLETS

Droplets with Membranes

Some of the first experiments on cytoplasmic streaming appeared in a brief report by Donné in 1838 (37), followed by a report by a "commissaire," Dutrochet (39), who witnessed and then repeated and somewhat extended Donné's results (38). Donné pressed some cytoplasm out of a *Chara* internodal cell and observed both in an isolated endoplasmic droplet and in intact cells the rotation of "green globules," i.e. single chloroplasts. On the basis of this result, Donné and the reviewers of his work concluded that chloroplasts were endowed with the power of motility. A chance observation of Dutrochet (39), duly witnessed by an eminent physician, showed that a chain of chloroplasts could either move in a circle, or if impeded could force the adjacent fluid to flow.

Donné (37) also wrote (translated from the original French):

> "One feels that I should have found out if there existed vibratile cilia at the surface of the (chloroplasts) endowed with spontaneous movement that I have just described, but so far my efforts have been in vain, and I have without success employed a magnification of 500 diameters and the best illumination; . . . (yet) I have not been able to assure myself of the existence of a ciliary apparatus; I thought I could see a bright halo around the (chloroplasts) but could not affirm more in this regard."

Jarosch (72–75) discovered these accounts in the early botanical literature and repeated the experiments by stripping cytoplasm from large characean internodal cells in its own cell sap. The cytoplasm formed membrane-bounded droplets in which various kinds of motility were observed, many of which were identical or related to movements in intact cells. Jarosch (72) confirmed Donné's report of the rotation of chloroplasts and showed that nuclei often do the same. He also demonstrated the presence of very fine filaments in the form of serpentine strands, closed circular and elliptical loops, and polygons with three to eight sides. He also noted (73, 74) that some but not all loops and polygons had associated particles that moved in a stream counter to the motion of the loop or polygon itself. Jarosch (75) also made detailed observations on intact internodal cells of *Nitellopsis stelligera,* and it is evident from his sketches that he probably saw the fibrils that connect chloroplasts in rows and the movement of particles along these. He correctly surmised that the fibrillar loops were either derived from or related to the fibrils that connect the chloroplasts. These points were later clearly demonstrated by Kamitsubo (78, 80) in photomicrographs and films.

Isolated Endoplasm

Kamiya & Kuroda (86) designed a special chamber in which characean cells could be subjected to a negative pressure of about 2 cm of Hg so that their turgor

pressure would not cause them to collapse if cut near one end extending through a vaseline seal outside the chamber. When this is done, a column of endoplasm streams out of the cut cell and forms a large droplet. When such a droplet was viewed from the bottom, many rotating chloroplasts could be seen. By using this technique, Kuroda (98) prevented the rotation of chloroplasts with a micromanipulator needle and found that a counter stream of particles around the chloroplast continued to move. She also observed the loops and polygons discovered by Jarosch and showed that these could be reversibly deformed by micromanipulation. Kuroda (98) also contributed the first quantitative data on the movements of these loops and polygons in isolated cytoplasm.

Kamitsubo (77, 81) observed the formation of polygons by the bending and fusion of straight fibrils (probably subcortical fibrils) at sharp angles. Thus it appears that the loops and polygons are derived from structures normally present in the cell. The work of Kamiya & Kuroda (86, 98) on isolated endoplasmic droplets clearly set the stage for the discovery of endoplasmic filaments (5), because it was demonstrated that endoplasm either contains vast numbers of motile fibrils or can generate these quickly after isolation from the cell.

Kamitsubo (79) later discovered that many more of these motile fibrils could be studied in the clear zones of centrifuged *Nitella flexilis* internodal cells. Furthermore, these cells survived indefinitely, whereas endoplasmic droplets are fragile. Kamitsubo's very penetrating study contains a wealth of information about motile fibrils.

The experiments on motile fibrils in endoplasmic droplets during the 1950s and 1960s sensitized workers in the field to the possible roles that filaments might play in streaming. In particular, these studies suggested that the transport of particles along filaments, i.e. saltation, might be part of the basic mechanism of streaming. The fact that serpentine filaments can translate through the cytoplasm and that all configurations of filaments can propagate bending waves (72, 73) led Jarosch (75) and Allen (5) to look for bending waves as part of the mechanism of streaming in intact cells.

Preservation of Motile Structures in Physiological Solution

Kamiya (84) has recently given a preliminary report on partially successful attempts to preserve the motility of rotating chloroplasts in vitro. A buffered, low-calcium medium that contains ATP prevents membranes of cytoplasmic droplets from reforming. As the cytoplasm gradually disperses into the medium, the chloroplast rotation continues almost as actively as in the intact droplet for up to 2 hr.

PHYSIOLOGICAL EXPERIMENTS ON INTACT CELLS

By now it should be clear that nearly all experiments on streaming in green plants have been executed on characean cells. It is important to find out to what extent the motile and regulatory mechanisms found in plant cells are analogous to those in other cells and especially muscle, and to what extent they are unique. We

hope that much of what is learned about characeans will elucidate streaming mechanisms not only in plants but in other systems as well.

The reviews of Kamiya (82, 83), which discuss the classical physiological experiments up to that time, and the present review overlap somewhat. This seems justified since at the time of these reviews Kamiya interpreted his results entirely on the basis of the active shearing theory. The present discussion considers from a broader perspective the experimental work on characean cells that appears relevant to the question of how the motive force for streaming is developed.

Cortical Injury

MECHANICAL Dutrochet (38, 39) was probably the first to point out that mechanical injury to the cell, manifested as a disruption of the spiral rows of chloroplasts, results in a disruption of the pattern of cytoplasmic streaming. Kamiya's reviews (82, 83) list other papers that confirm that observation. Kamiya and earlier authors have stressed one interpretation of the effect of local injury: that the motive force would appear to be applied at the border of the cortex and endoplasm of the cell. The discovery of endoplasmic filaments (5) now requires a reevaluation of that interpretation because the filaments, being branches of the subcortical fibrils, would also be disrupted by any injury that affects the subcortical fibrils.

CENTRIFUGAL ACCELERATION Although it was Linsbauer (106) who first showed that centrifugation simultaneously dislodges patches of chloroplasts and disrupts streaming, Hayashi (56, 57) was the first to study its effects systematically and observe stages in the recovery of streaming after injury. He showed that cytoplasm accumulates in recently injured regions, but that gradually 20 hr after injury, the streaming becomes reestablished.

MICROBEAM OF LIGHT Kamitsubo (80) has found perhaps the best way to control cortical injury. A selected region of the cell is transilluminated by a microbeam ca 100 μm in diameter from an unfiltered mercury arc (HB 200) lamp. The chloroplasts first bleach, then swell, and are finally loosened and carried away by the streaming cytoplasm. The area denuded of chloroplasts may show initially very little injury in some cases, although in other cases the subcortical fibrils may be displaced toward the vacuole or even destroyed (N. S. Allen, personal observation). In the latter case, cytoplasm accumulates over the site of injury until the subcortical fibrils are restored (78). Often this stagnant cytoplasm is highly light scattering. Within a few days the fibrils and endoplasmic filaments become reestablished, and streaming is normal, both microscopically and as viewed by laser-Doppler spectroscopy.

INSTANTANEOUS ACCELERATION Kamiya & Kuroda (89) placed *Nitella* internodal cells into a chamber inside a steel hammer, which was then driven repeatedly as rapidly as possible against an anvil, which caused repeated instantaneous accelerations of 4,000–7,000 $\times$ g for 0.06–0.14 msec. These were capable of dislodging portions of the cortical layer. The injury in the case of instantaneous acceleration

was quite different from that produced by prolonged centrifugal acceleration, which removed and sedimented entire patches of cortex. Instantaneous acceleration instead loosens chloroplasts in rows, which then peel off singly or several at a time in response to the flow of cytoplasm under the rows. Kamiya & Kuroda (89) showed that not only single chloroplasts, but rows of chloroplasts have an independent motility in cytoplasm. It is interesting to note that Dutrochet (38) observed similar phenomena in 1838. The hammer experiments have been repeated recently (N.S. Allen, personal observation) and observations made with a sensitive differential interference contrast microscope indicated that the chloroplasts were held in rows by the firmly attached subcortical fibrils, from which some waving branches could be seen. It seems probable, therefore, that the motility of single chloroplasts and rows of chloroplasts is due to the activities not only of segments of subcortical fibrils attached to the chloroplast rows, but also to what is left of their branches, the endoplasmic filaments.

Ligation and Compression

Donné's remarkable study 140 years ago (37) reported the use of Purkinje's microcompressor in experiments that detached rows of chloroplasts that showed independent motility. The same year, Dutrochet (38) performed the first ligation experiment on a characean cell. Since that time, these procedures have served various experimental purposes. Hayashi (55), for example, showed how a combination of centrifugation and ligation could produce a vacuole-free cell. Linsbauer (106) used compression to alter the pattern of streaming.

The most elegant use of controlled compression has been that of Kamiya & Kuroda (91), who demonstrated that although the viscous resistance of the vacuolar sap is negligible, streaming slows down if the cell is compressed so that the opposite streams of endoplasm mingle. The purpose of the experiment was to measure the motive force by opposing it with endoplasmic viscous resistance (which was measured separately as a force-flow diagram). As expected, the compression of the cell decreased the streaming velocities. However, it is not clear whether this occurred because of viscous resistance or as the result of mechanical interference between opposing sets of endoplasmic filaments. Assuming the active shearing model to be correct, Kamiya & Kuroda (91) were able to estimate the motive force as approximately 1.7 dyn cm^{-2}, a value very similar to measurements by other methods that assume the same theory of streaming is correct. This same paper contains a force-flow diagram measured for endoplasm removed by the negative pressure technique. Although we do not know whether all or only some of the endoplasmic filaments remain with these large drops of endoplasm when isolated, the force-flow diagram shows a dramatic difference in apparent viscosity at different rates of shear below 5 sec^{-1}. If this non-Newtonian behavior of isolated cytoplasm is due to the presence of endoplasmic filaments, then this curve gives some idea of the lability of filaments under conditions prevalent in the cell. It is unfortunate that these authors did not explore the viscosity and birefringence at higher shear rates and perform the measurements in the reverse order to look for hysteresis and thixotropic behavior.

Chemical Facilitation and Inhibition of Streaming

Experiments on the effects of stimulating and inhibiting substances on streaming are very difficult to interpret because the agents are rarely as specific as hoped when the experiments were performed, and we know so little about the chemical mechanisms of streaming that it is difficult to decide which experiments are relevant.

ADENOSINE TRIPHOSPHATE ATP is a case in point. Sandan & Somura (144) found that 10^{-3} M ATP would accelerate streaming by 15% in *Nitella flexilis*. On the other hand, Hayashi (57) found no effect in *Chara braunii*. By using laser-Doppler spectroscopy, Mustacich & Ware (117) found that ATP had two effects: on velocity of streaming and on release of particles. A similar result has also been reported in cytoplasmic perfusion models (179). The results of Nachmias (118) and of Taylor et al (155) on amoebae clearly show that experiments regarding ATP effects on streaming are without meaning unless one knows the concentrations of divalent ions present and the effects of ADP, AMP, and inorganic pyrophosphate controls. Furthermore, it is important to know whether the ATP is bound to the cell surface or enters the cell. Therefore, much of the older work on the effects of ATP (82, 83) is uninterpretable.

COLCHICINE AND OTHER MICROTUBULE POISONS Colchicine binds specifically 6S tubulin dimers and inhibits their assembly into microtubules (19, 20). Vinblastine also binds to tubulin and precipitates it as microtubular paracrystals. Either of these drugs will interfere with microtubule-associated motility or transport processes.

Colchicine disrupts the microtubules of characeans, which causes profound changes in cell shape (50). However, streaming continues unaffected. In general, microtubule assembly inhibitors have little effect on streaming in plant cells. However, there are notable exceptions; in *Acetabularia,* nuclei are inhibited from forming normal cysts in forming caps. In *Ulva,* rhythmic chloroplast displacements are inhibited by colchicine after a delay of one cycle, whereas cytochalasin B had no effect (23).

CYTOCHALASINS A AND B Cytochalasins A (CA) and B (CB) are fungal metabolic products that have an inhibiting effect on some but not all actin-based motile systems. The action of these substances are varied, and in only a few cases is anything known about the chemical mechanism of actin. CB is known to bind to membranes (154) and to affect membrane transport (134), the viscosity of F-actin preparations polymerized in its presence (150), gel formation by actin (175), and the binding of actin-binding protein to actin (51, 52). As yet no data exist on possible chemical mechanisms of its action on plants.

CA inhibits the self assembly of beef brain tubulin and muscle G actin in vitro and decreases the ability of tubulin to bind colchicine. CA evidently exerts its action on sulfhydryl groups (60). CA may be present as an impurity in CB.

The effects of CB on motility are not understood. Some indirect evidence suggests

it associates with myosin (92, 123). Tannenbaum et al (154) suggest that binding to the plasma membrane could mean binding to submembranous actin.

Whatever the mechanism of its action may be, there is no doubt that CB inhibits streaming reversibly in *Nitella* and *Chara* within 15–20 min of its application in concentrations of 1–50 $\mu g\ ml^{-1}$ (21, 177). There are still microfilaments present with CB (21).

An example of a more rapid effect is seen when 5–25 μg of CB per ml is applied to *Mougeotia* cells. Within seconds, all saltations cease, and no photodinesis occurs in response to changes in light intensity (171; N. S. Allen, personal observation).

HALOGENATED BENZOIC ACID DERIVATIVES Brueske & Applegate (24) reported that ortho-substituted benzoic acids had an accelerating action on streaming in *Elodea,* whereas parasubstituted compounds had an inhibiting effect. It is suggested that streaming may be affected by two pathways: one accelerating and the other inhibiting. Since there are few chemical compounds the accelerate streaming, this finding seems interesting, but not yet interpretable.

SULFHYDRYL INHIBITORS Abé (1) reported that *p*-chloromercuribenzoate, a highly specific —SH reagent, had a strong inhibiting effect on cytoplasmic streaming of several types. The inhibition was reversed by treatment with cysteine. Abé's paper records the only known case of the reversal of direction of streaming in *Nitella.*

Another thiol inhibitor, N-ethylmaleimide (NEM), has a similar effect on streaming except that the inhibition is irreversible (26).

Differential Treatment of the Cortex and Endoplasm with Inhibitors

Chen & Kamiya (26) have introduced an ingenious method for differential treatment of two halves of a centrifuged *Nitella* internodal cell in two separate chambers. The cell is centrifuged in a double-chambered cuvette in which either the centrifugal or centripetal half of the cell can be treated selectively with an inhibitor. They found that treatment of the centrifugal half (containing the endoplasm) with the inhibitor, NEM, caused an irreversible loss of motility. However, in the opposite experiment, treatment of the cortex alone in the centripetal half of the cell with NEM did not inhibit streaming once the inhibitor was removed. The interpretation of the results, suggested by the title of the paper, "Localization of Myosin in the Internodal Cell of *Nitella* as suggested by Differential Treatment with N-Ethylmaliemide," is based on the fact that actin-activated muscle myosin Mg^{2+} ATPase activity is inhibited irreversibly by NEM. Now that myosin has been isolated and characterized in *Nitella,* its specific sensitivity to NEM needs to be established to clarify this experiment. There are two additional problems with this approach. First, since the endoplasmic layer is normally about 10 times thicker than cortex, its volume is considerably greater. The experiment of Chen & Kamiya (26) did not measure the amounts of NEM bound in the two halves of the cell in reciprocal experiments. Thus they have not excluded the possibility of differential doses admin-

istered to the two halves because of differential uptake or binding. Second, there may be many components in the motile apparatus of the cell that contain functionally important —SH groups that are attacked by NEM. Thus the specificity of the inhibitor for the assumed myosin is not established. However, from other evidence, we believe that myosin is located in the endoplasm.

A similar differential treatment protocol has been followed with the drug CB. In this case, the opposite result was found. CB treatment of the cortex inhibited streaming in that half of the cell, whereas CB treatment of the half that contains the endoplasm had no effect. Nagai & Kamiya (118a) also centrifuged *Nitella* cells, exposed them to CB and cytachalasin D, and obtained similar results to those obtained in (26), namely, that no streaming occured in the endoplasm when the cortex was CB treated, whereas when the endoplasm was treated with CB and the cortex was not, streaming was near normal. The authors state that the CB likely inhibits the microfilaments, specifically those in the subcortical fibrils. It is clear that CB has an effect on the cortex. Further, they conclude that motile filaments in the endoplasm play no part in streaming. We doubt whether this conclusion is valid, since it conflicts with the direct observation that endoplasmic filaments transport particles in a manner similar to the subcortical fibrils.

Perhaps the answer to the question depends on an interaction of B and some actin-binding proteins (51, 52). Ca^{2+} and CB affect actin-binding proteins, as shown by Condeelis & Taylor (28). We already know that in perfused *Nitella* cells no movement occurred when cytochalasin and ATP were applied simultaneously, and at the same time particle release was inhibited. Furthermore, Ca^{2+} inhibited the streaming. The precise relationships of these events must await biochemical analysis. This may be an important result, but we cannot interpret it until the specific effects of CB on these algal cells is known. This question must be posed at the biochemical level, and as can be seen from the earlier discussion of CB we do not yet have sufficient information to know what CB affects. For these reasons, it is necessary to reserve judgment on the significance of these experiments and the conclusions drawn from them.

Electrical and Mechanical Stimulation

Hörmann (64) studied the effects of electricity on streaming and discovered the charophyte action potential, which was first measured by Blinks, Harris & Osterhout (18) in *Nitella.* Characeans are excitable cells and conduct an electrical depolarization along their length. Concomittantly, streaming stops temporarily and resumes only gradually, reaching peak velocity in 5–20 min. The electrophysiology of characean cells is reviewed by Hope & Walker (63), and its relation to streaming is discussed by Kamiya (82). Although the point has been the subject of controversy in the past, it is now clear that the action potential stops streaming by turning off the motive force (157) and not by causing the cytoplasm to gelate, as was once believed. In fact, after stimulation, Brownian motion occurs throughout the endoplasm (96, 169). Allen (5) took advantage of this fact to measure the endoplasmic microviscosity in *Nitella.*

The effect of the action potential on the motive force appears to be mediated

by the ionic composition of the medium. Chloride ions penetrate (30), but have been shown to have little effect on streaming (179). By using vacuolar perfusion, the cell sap of *Nitella* was exchanged for various ionic media. It was found that concentrations of Ca^{2+} in excess of 50 mM cause cyclosis to slow down (160). When calcium was replaced by Mg^{2+}, the endoplasm streamed normally.

Exposing *Nitella* cells to Ca^{2+}, Sr^{2+}, Mg^{2+}, and Ba^{2+} chlorides as the sole electrolyte in the external solution, Barry (15) found that the action potential increased in the order Ca < Sr < Mg < Ba. Excitation caused streaming to stop only in $CaCl_2$ or $SrCl_2$, and a Ca^{2+}/Mg^{2+} ratio of 1/20 was required to halt streaming. Thus it is not the action potential itself that halts streaming but the action of calcium ions. Curiously, in perfused Characean cells that have no tonoplast, cytoplasmic streaming is neither slowed nor stopped during action potentials (158a). The EGTA must rapidly remove the Ca^{2+}.

It would appear likely that Ca^{2+} enters on stimulation to cause streaming to halt, because Williamson (179) has shown with tonoplast-free models that streaming continues in the presence of Mg^{2+} but stops when Ca^{2+} ($>10^{-6}$ mM) is administered. Similarly, in Characean cells with the tonoplast removed, ATP and Mg^{2+} must be present for streaming to occur (158a).

On either mechanical or electrical stimulation of *Nitella* cells, straight endoplasmic filaments are seen from the cortex to the tonoplast. The first sign of the resumption of streaming is saltations along these filaments and along the subcortical fibrils (5, 6).

Vacuolar Perfusion

Characean and certain other large algal cells (e.g. *Acetabularia*) lend themselves to a vacuolar perfusion technique developed especially for electrophysiological studies (35, 158, 160–162). Both ends of a cell are excised in a special chamber and the natural vacuolar sap is replaced. If the medium contains the right ionic composition, streaming continues. For example, after ligation, a perfused Characean cell can survive for many days in artificial pond water. If insufficient calcium is present in the perfusate, streaming becomes slower and the cell eventually dies (158).

Another useful technique is the "open vacuole" method in which the ends of the cell are not ligated. It has been used to measure membrane potentials and resistances in *Nitella*.

Cytoplasmic Perfusion

Recently, Tazawa et al (158a, 159) found it possible to remove the tonoplast of a *Nitella* or *Chara* cell by perfusing with EGTA-containing media. Most of the endoplasm tends to be washed away at the same time. Cytoplasmic perfusion was used by Williamson (179) for a careful study on the ionic and biochemical requirements for particle movement along subcortical fibrils (fibers). By perfusing a low-calcium medium [$(Ca^{2+}) < 10^{-7}$ M], with K^+ and sucrose as osmotica, he was able to show that against the stream only particles moving in the vicinity of the subcortical fibrils continued in the original direction whereas endoplasm was

reversed by the perfusion. By the time most of the endoplasm was washed away by perfusion, the remaining particles had come to rest on the fibrils. The addition of ATP then caused the particles to resume movement, but it also caused them to be released from the fibrils. Pyrophosphate did not promote movement, but did release the particles. This is the result one would expect if a myosin served to bind the particles to the fibrils. No calcium requirement was found, as in the amoeba experiments after which these were designed (155), and 10^{-5} M Ca^{2+} was found to be 80% inhibitory. Magnesium, however, was required. If cytochalasin and ATP were supplied simultaneously, no movement occurred and particle release was inhibited. The CB effect was partially reversible. It is well known that in muscle and most nonmuscle motile systems, calcium plays a regulatory role. Therefore, it is curious at first sight that calcium plays an inhibitory role in characean cells. However, in a steady-state process like rotational streaming, it may be an interesting adaptation that the penetration of calcium leads to temporary cessation of movement. The recent findings on myosin ATPases (91a) should be kept in mind along with these facts when trying to understand the control of streaming in *Nitella.*

Williamson's interesting study is important because it established the basic ionic and biochemical requirements for the saltation or "active shearing" interaction that propels particles along bundles of F-actin filaments. However, a recent discussion of the theoretical aspects of characean streaming by Williamson (180) can be criticized for not weighing heavily enough the observations on saltations that occur on filaments throughout the endoplasm (5, 6) that oppose the active shearing theory in its original form. For example, Donaldson's (35) calculations in support of the contention that subcortical fibrils alone can move the entire endoplasm are irrelevant if the endoplasm contains, as it does, vast numbers of endoplasmic filaments that transport particles and fluid.

Estimates of the Motive Force

Three "independent" methods have been devised for "measuring" the motive force for rotational streaming: (*a*) balance acceleration (87); (*b*) vacuolar perfusion (35, 157); and (*c*) viscous resistance by compressing two opposing endoplasmic streams until they join (90, 91). The shared factor in all of these methods that makes them less independent than it would initially seem is the assumption common to all three that the motive force is applied by the subcortical fibrils. All of these methods yield values in the range of 1–4 dyn/cm^2.

Since endoplasmic filaments both undulate and provide a substratum for saltatory movements throughout the endoplasm, any of the forces used in these three methods would interfere with endoplasmic filament activities and thus reduce the force they were intended to measure (5).

On the hypothesis that the undulation of endoplasmic filaments might propel rotational streaming, Allen used standard hydrodynamic equations to estimate the total force they would exert if actively undulating. The result was a value of 10 dyn/cm^{-2}. We are inclined to believe that even the latter figure may be a lower limit because the saltations along endoplasmic filaments are not included

in any estimate, and if the observed undulations are not active, they must represent a form of dynamic viscous resistance that is abolished by all three compensation techniques.

THEORIES OF CYTOPLASMIC STREAMING

It is remarkable that the early 19th century French scientists Donné (37) and Dutrochet (38, 39) arrived at a state of understanding of cytoplasmic streaming in characean cells that was so nearly equivalent to our own. Although they knew little or nothing about chloroplasts and their role in photosynthesis, they found out that the mechanism of streaming was closely tied to the "green globules" because they showed independent rotational motility. They even knew that some structure held the chloroplasts together in rows, and that a row of chloroplasts could show independent translational motility. These facts were forgotten and not generally known in the present century until Jarosch (72–75) rediscovered these interesting papers and repeated and extended their findings.

In 1929, Linsbauer (106) conducted many careful experiments that led him to believe that the motive force was produced by an unspecified interaction between the streaming endoplasm and the stationary cortex.

Kamiya & Kuroda (85) confirmed this notion by their velocity profiles, which they interpreted to indicate that shear occurred only at the border between the endoplasm and the cortical layer. The velocity profiles were approximately correct, but since the presence of endoplasmic filaments was unsuspected, it could not have been forseen that shear occurs throughout the endoplasm as well as at the surface of every endoplasmic filament. In accordance with prevailing concepts (111), it was proposed that the motive force was an active shearing that somehow developed at the border between a sol and a gel; that is, at the border between the streaming endoplasm and the stationary cortex (85). Donaldson (36) elaborated this idea by suggesting that oscillations at the sol-gel border might produce the motive force.

Jarosch's important extension of the observations of the early French workers focused attention on the possible role of "motile fibrils" in the streaming process (72–75). It is evident from his description and drawing that Jarosch (75) observed subcortical fibrils and saw particles saltate along them, but it was left to Kamitsubo (77) to document their presence and show clearly that they constituted at least part of the motive force system for rotational streaming.

Nagai & Rebhun (119) showed that the subcortical fibrils were bundles of 50- to 70-Å microfilaments and suggested that they might be actin.

The demonstration of HMM binding by subcortical fibrils (93, 126, 178) established that the subcortical fibrils are bundles of actin. In the early 1970s the prevailing view was that all of the motive force was delivered in the vicinity of the subcortical fibrils by an interaction of a myosinlike mechanoenzyme, either associated with particles (138, 146) or dispersed in the ground substance. It was assumed that the rheological properties of the endoplasm would permit it to be carried as a plug by this interaction at the inner surface of the cortex. This has been restated in hydrodynamic terms by Donaldson (35).

The discovery of endoplasmic filaments (5) along which particles saltate at streaming velocity, and which undulate sinusoidally, has required a reevaluation of the active-shearing theory and at the same time has suggested several new testable hypotheses.

It now seems clear that the interaction between particles (especially sphaerosomes and mitochondria) and either subcortical fibrils or endoplasmic filaments is the crux of what Kamiya & Kuroda (85) referred to as active shearing. It is equally clear that this process can account for the transport of particles not only at the inner surface of the cortex, but throughout the endoplasm.

Endoplasmic streaming carries not only the visible particles, but the fluid in which they are suspended. It is entirely possible that the fluid moves as the result of drag forces that the totality of moving particles apply to the ground cytoplasm in which they move. Allen (5) observed the initiation of streaming in the subcortical and endoplasmic regions of a cell after stimulation. In both regions the events were the same: single rapid particle saltations along endoplasmic filaments and subcortical fibrils first, then more frequent saltation, followed by a gradual "catching up" of the surrounding small particles. Such a modified form of the active shearing theory may prove to be adequate to account for rotational streaming.

The fact that filaments show sinusoidal undulation in *Nitella* cells might be given two opposite interpretations. Allen (5) preferred to consider the proposition that the undulation might be active. This is a reasonable working hypothesis, because sinusoidal bending waves are well known to provide the propulsive force for thousands of protists and sperm of many kinds.

Hydrodynamic calculations are never very precise when dealing with fluids with a complex rheology; nevertheless, it was of more than passing interest that streaming velocity could be predicted within a factor of three, and that the motive force estimated (9.9 dyn/cm^2) was actually well in excess of experimental estimates. (We are reluctant to use the term "measurements" because each method used assumes that the motive force is active shearing and therefore is not a nonintrusive measurement.) These estimates show only that the undulations if due to active bending waves, produce sufficient force to drive the endoplasm around the cell. They do not prove that the bending is active.

The opposite hypothesis is that the bending is a passive process. In the same way that an unbalanced wheel can cause an axel to show vertical oscillations, it is reasonable to expect that an unbalanced load of saltating particles, and the unbalanced drag force they might produce, could cause thin filaments to undulate in a sinusoidal waveform determined by the imbalanced load and the stiffness of the filament. According to the passive undulation hypothesis, the force required to bend the filaments would consume some fraction of the motive force that causes the cytoplasm to stream. At the present time no evidence exists that would permit us to decide between these two hypotheses.

The molecular basis of active shearing and filament undulation is still uncertain. There seems to be no doubt that the subcortical fibrils and endoplasmic filaments are composed in part of F-actin filaments. Schmitt (146) once proposed a general model for saltation in which a particle might move along an actin filament through the action of myosin molecules with their tails embedded in the particle membrane.

Kamiya (84) has proposed an idea similar to that of Williamson (180) and Schmitt (146), with some evidence in its favor (26). In support of this idea is the fact that the only myosinlike enzyme activity in *Nitella* extracts was localized in the particulate fraction of *Nitella* (6).

The active shearing theory (modified version) applies very well to what we know of the other types of streaming in green plant cells. In fact, rotational and multistriate streaming (e.g. *Acetabularia, Phycomyces*) become practically identical in principle. Saltation, circulation, fountain, and reverse-fountain streaming also become comprehensible in this way, although there is no evidence to confirm this idea.

It is not yet known whether or not filament undulation is a general phenomenon in plant cytoplasm, and improved optics are probably required to determine whether it is. One might expect to find undulating filaments in phloem cells (44) and in nerve axons, perhaps. It is quite possible that filament undulation, if it is an active process, evolved as a mechanism for transporting the large quantities of endoplasm rapidly in these cells.

ACKNOWLEDGMENTS

We acknowledge the use of the library facilities and the expert help of the staff of the Marine Biological Laboratory, Woods Hole, Mass. The preparation of this review was funded in part by Grant GM 22356 from NIH.

Literature Cited

1. Abé, S. 1964. *Protoplasma* 58:483–92
2. Aikman, D. P., Anderson, W. P. 1971. *Ann. Bot.* 35:761–72
3. Allen, N. S. 1973 *Endoplasmic filaments generate the motive force for rotational streaming in Nitella.* PhD thesis. Univ. of Md., College Park. 66 pp.
4. Allen, N. S. 1973. *Endoplasmic filaments in Nitella translucens.* Film, obtainable at cost from Calvin Communications, Inc., 1105 Truman Rd., Kansas City, Mo 64106
5. Allen, N. S. 1974. *J. Cell Biol.* 63:270–87
6. Allen, N. S. 1976. In *Cell Motility,* Cold Spring Harbor Conf. Cell Proliferation, ed. R. Goldman, T. Pollard, J. Rosenbaum, pp. 613–21. Cold Spring Harbor: Cold Spring Harbor Lab. 1373 pp.
7. Allen, N. S., Allen, R. D., Reinhart, T. 1976. *Biol. Bull.* 151:398 (Abstr.)
8. Allen, R. D. 1960. *J. Biophys. Biochem. Cytol.* 8:379–97
9. Allen, R. D. 1961. In *The Cell,* ed. J. Brachet, A. E. Mirsky, Vol. II, pp. 135–216. New York: Academic. 916 pp.
10. Allen, R. D. 1964. In *Primitive Motile Systems in Cell Biology* ed. R. D. Allen, N. Kamiya, pp. 407–32. New York: Academic. 642 pp.
11. Allen, R. D. 1977. In *International Cell Biology,* ed. B. R. Brinkley, K. R. Porter, pp. 403–06. New York: Rockefeller Univ. Press. 694 pp.
12. Allen, R. D., Allen, N. S. 1974. *Photodinesis.* New York: Harper and Row (Film)
13. Allen, R. D., Allen, N. S. 1978. *Ann. Rev. Biophys. Bioeng.* 7:469–95
14. Anderson, W. P. 1974. *Symp. Soc. Exp. Biol.* 28:63–85
15. Barry, H. 1968. *J. Cell Physiol.* 72:153–60
16. Berthold, G. 1888. *Studien über Protoplasmamechanik.* Leipzig: Arthur Felix. 322 pp.
17. Biddulph, O. 1941. *Am. J. Bot.* 28:348–52
18. Blinks, L. R., Harris, E. S., Osterhout, W. J. V. 1929. *Proc. Soc. Exp. Biol. Med.* 26:836–38

19. Borisy, G. G., Taylor, E. W. 1967. *J. Cell Biol.* 34:525–33
20. Borisy, G. G., Taylor, E. W. 1967. *J. Cell Biol.* 34:535–48
21. Bradley, M. O. 1973. *J. Cell Sci.* 12:327–43
22. Breckheimer-Beyrich, H. 1954. *Ber. Dtsch Bot. Ges.* 67:86–92
23. Britz, S. J. 1976. *Carnegie Inst. Washington Yearb.* 75:392–94
24. Brueske, C. H., Applegate, H. G. 1962. *Phyton* 18(1):27–32
24a. Bünning, E. 1977. *Ann. Rev. Plant Phys.* 28:1–22
25. Canny, M. J. 1974. *Phloem Translocation.* Cambridge, England: Cambridge Univ. Press. 301 pp.
26. Chen, J. W., Kamiya, N. 1975. *Cell Struct. Funct.* 1:1–9
27. Condeelis, J. S. 1974. *Exp. Cell Res.* 88:436–39
28. Condeelis, J. S., Taylor, D. L. 1977. *J. Cell Biol.* 74:901–27
29. Corti, B. 1774. *Osservazione Microscopische sulla Tremella e sulla Circulazione del Fluido in Una Planto Acquaguola.* Lucca, Italy
30. Coster, H. G. L. 1966. *Aust. J. Biol. Sci.* 19:545–54
31. Crafts, A. S., Crisp, C. E. 1971. *Phloem Transport in Plants.* San Francisco: Freeman. 481 pp.
32. Cronshaw, J., Esau, K. 1967. *J. Cell Biol.* 34:801–16
33. Dempsey, G. P., Bullivant, S., Bieleski, R. L. 1976. In *Transport and Transfer Processes in Plants,* ed. I. F. Wardlaw, J. Passionara, pp. 247–51. New York: Academic. 484 pp.
34. DeVries, H. 1885. *Bot. Z.* 43:1–6; 16–26
35. Donaldson, I. G. 1972. *Protoplasma* 74:329–44
36. Donaldson, I. G. 1972. *J. Theor. Biol.* 37:75–91
37. Donné, A. 1838. *Ann. Sci. Nat. Bot. Biol. Veg.* 10:346–48
38. Dutrochet, M. 1838. *Ann. Sci. Nat. Bot. Biol. Veg.* 9:5–38
39. Dutrochet, M. 1838. *Ann. Sci. Nat. Bot. Biol. Veg.* 10:349–55
40. Edds, K. T. 1975. *J. Cell Biol.* 66:145–55
41. Edds, K. T. 1975. *J. Cell Biol.* 66:156–64
42. Ewart, J. 1903. *On the Physics and Physiology of Protoplasmic Streaming in Plants.* New York: Oxford Univ. Press. 131 pp.
43. Fensom, D. S. 1972. *Can. J. Bot.* 50:479–97
44. Fensom, D. S., Williams, E. J. 1974. *Nature* 250:490–92
45. Forer, A., Jackson, W. T. 1975. *Cytobiology* 10(2):217–26
46. Forer, A., Jackson, W. T. 1976. *Cytobiology* 12(2):199–214
47. Gilder, J., Cronshaw, J. 1973. *J. Ultrastruct. Res.* 44:388–404
48. Goldman, R. D., Knipe, D. M. 1972. *Cold Spring Harbor Symp. Quant. Biol.* 37:523–34
49. Goldman, R. D., Pollard, T. D., Rosenbaum, J. L. 1976. *Cell Motility,* Cold Spring Harbor Conf. Cell Proliferation, Vols. I, II, III. Cold Spring Harbor: Cold Spring Harbor Lab. 1373 pp.
50. Green, P. G. 1969. *Ann. Rev. Plant. Physiol.* 20:365–94
51. Hartwig, J. H., Stossel, T. P. 1975. *J. Biol. Chem.* 250:5696–705
52. Hartwig, J. H., Stossel, T. P. 1976. *J. Cell Biol.* 71:295–303
53. Haupt, W. 1962. In *Physiology and Biochemistry of Algae.* ed. R. A. Lewin, pp. 567–72. New York: Academic. 925 pp.
54. Haupt, W., Schönbaum, E. 1970. In *Photobiology of Microorganisms,* ed. P. Halldal, pp. 283–307. London: Wiley. 479 pp.
55. Hayashi, T. 1952. *Bot. Mag.* 65:51–55
56. Hayashi, T. 1957. *Bot. Mag.* 70:168–74
57. Hayashi, T. 1960. *Sci. Pap. Coll. Gen. Ed. Univ. Tokyo* 10:245–82
58. Heilbrunn, L. V. 1958. *Protoplasmatologia* 2(C1):1–109
59. Hepler, P. K., Palevitz, B. A. 1974. *Ann. Rev. Plant Physiol.* 25:309–62
60. Himes, H. H., Houston, L. L. 1976. *J. Supramol. Struct.* 5:57–64
61. Hiromoto, Y. 1970. *Biorheology* 6: 201–34
62. Hitchcock, S. E. 1977. *J. Cell Biol.* 74:1–15
63. Hope, A. B., Walker, N. A. 1975. *The Physiology of Giant Algal Cells.* Cambridge England: Cambridge Univ. Press. 201 pp.
64. Hörmann, G. 1898. *Studien über die Protoplasma-strömung bei der Characeen.* Jena: Fischer
65. Huxley, H. E. 1969. *Science* 164:1356–66
66. Ilker, R., Currier, H. B. 1974. *Planta* 120:311–16
67. Inoué, S., Stephens, R. E. 1975. *Molecules and Movement,* Soc. Gen. Physiol. Ser., Vol. 30. New York: Raven Press. 450 pp.

68. Ishikawa, H., Bischoff, R., Holtzer, H. 1969. *J. Cell Biol.* 43:311–16
69. Iwanami, Y. 1956. *Phytomorphology* 6:288–95
69a. Jackson, W. T., Doyle, B. G. 1977. *J. Cell. Biol.* 75:268a
70. Jahn, T. L., Rinaldi, R. A. 1959. *Biol. Bull.* 117:100–18
71. Jarosch, R. 1956. *Protoplasma* 47:478–86
72. Jarosch, R. 1956. *Phyton* 6:87–107
73. Jarosch, R. 1957. *Biochim. Biophys. Acta* 25:204–5
74. Jarosch, R. 1958. *Protoplasma* 50:94–108
75. Jarosch, R. 1960. *Phyton* 15:43–66
76. Johnson, R. P. C. 1968. *Planta* 81:314–32
77. Kamitsubo, E. 1966. *Proc. Jpn. Acad.* 42:507–11
78. Kamitsubo, E. 1966. *Proc. Jpn. Acad.* 42:640–43
79. Kamitsubo, E. 1972. *Protoplasma* 74:53–70
80. Kamitsubo, E. 1972. *Exp. Cell Res.* 74:613–16
81. Kamitsubo, E. 1972. *Symp. Cell Biol.* 23:123–30
82. Kamiya, N. 1959. *Protoplasmatologia* 8(3a)1–199
83. Kamiya, N. 1962. *Encycl. Plant Phys. XVII* 2:979–1035
84. Kamiya, N. 1977. In *International Cell Biology Symposium,* ed. B. Brinkley, K. R. Porter, pp. 361–66. New York: Rockefeller Univ. Press. 694 pp.
85. Kamiya, N., Kuroda, K. 1956. *Bot. Mag.* 69:544–54
86. Kamiya, N., Kuroda, K. 1957. *Proc. Jpn. Acad.* 33:149–52
87. Kamiya, N., Kuroda, K. 1957. *Proc. Jpn. Acad.* 33:201–5
88. Kamiya, N., Kuroda, K. 1958. *Protoplasma* 50:144–48
89. Kamiya, N., Kuroda, K. 1964. *Ann. Rep. Sci. Works Osaka Univ.* 12:83–97
90. Kamiya, N., Kuroda, K. 1965. In *Symposium on Biorheology,* ed. A. L. Copley, Pt. 4, pp. 157–71. New York: Wiley. 634 pp.
91. Kamiya, N., Kuroda, K. 1973. *Biorheology* 10:179–87
91a. Kato, T., Tonomura, Y. 1977. *J. Biochem.* 82:777–82
92. Kemp, R. B., Jones, B. M., Gröschel-Stewart, U. 1973. *J. Cell Sci.* 12:631–39
93. Kersey, Y. M. 1974. *J. Cell Biol.* 63(2,II):165a (Abstr.)
94. Kersey, Y. M., Hepler, P. K., Palevitz, B. A., Wessells, N. K. 1976. *Proc. Natl. Acad. Sci. USA* 73:165–67
95. Kersey, Y. M., Wessells, N. K. 1976. *J. Cell Biol.* 68:264–75
96. Kishimoto, U., Akabori, H. 1959. *J. Gen. Physiol.* 42:1167–83
97. Kumagaya, S. 1950. *Bot. Mag.* 63: 52
98. Kuroda, K. 1964. In *Primitive Motile Systems in Cell Biology,* ed. R. D. Allen, N. Kamiya, pp. 31–41. New York: Academic. 642 pp.
99. Küster, E. 1935. *Die Pflanzencelle.* Jena: Fisher. 672 pp. 1st ed.
100. Küster, E. 1951. *Die Pflanzencelle.* Jena: Fisher. 2nd ed.
101. Küster, E. 1956. *Die Pflanzencelle.* Jena: Fisher. 3rd ed.
102. Kuźnicki, L., Sikora, J. 1971. *Acta Protozool.* VIII:439–46
103. Langley, K. H., Piddington, R. W., Ross, D., Sattelle, D. B. 1976. *Biochim. Biophys. Acta* 444:893–98
104. Lazarides, E. 1976. In *Cell Motility A,* Cold Spring Harbor Conf. Cell Proliferation, ed. R. Goldman, T. Pollard, J. Rosenbaum, Vol. 3, pp. 347–60. Cold Spring Harbor: Cold Spring Harbor Lab. 1373 pp.
105. Lee, D. R., Arnold, D. C., Fensom, D. S. 1971. *J. Exp. Bot.* 22:25–38
106. Linsbauer, K. 1929. *Protoplasma* 5:563–621
107. Lundegårdh, H. 1921. In *Handbuch des Planzenanatomie,* ed. Linsbauer, Bd. 1, II., pp. 351–80. Berlin. 402 pp.
108. MacRobbie, E. A. C. 1971. *Biol. Rev.* 46:429–81
109. Mahlberg, P. G. 1964. In *Primitive Motile Systems in Cell Biology,* ed. R. D. Allen, N. Kamiya, pp. 43–68. New York: Academic. 642 pp.
110. Marchant, H. J. 1976. *Planta* 131:119–20
111. Mast, S. O. 1926. *J. Morphol. Physiol.* 41:347–425
112. McGee-Russell, S., Allen, R. D. 1971. In *Advances in Cell and Molecular Biology I,* ed. E. J. Dupraw, pp. 153–84. New York: Academic. 308 pp.
113. Deleted in proof
114. Murmains, L., Evert, R. F. 1966. *Am. J. Bot.* 53:1065–78
115. Mustacich, R. V., Ware, B. R. 1974. *Phys. Rev. Lett.* 33:617–20
116. Mustacich, R. V., Ware, B. R. 1976. *Biophys. J.* 16:373–88
117. Mustacich, R. V., Ware, B. R. 1977. *Biophys. J.* 17:229–41
118. Nachmias, V. T. 1969. *J. Cell Biol.* 40:160–66

118a. Nagai, R., Kamiya, N. 1977 *Exp. Cell Res.* 108:231–37
119. Nagai, R., Rebhun, L. I. 1966. *J. Ultrastruct. Res.* 14:571–89
120. Nägeli, C. 1860. *Beitr. Wiss. Bot.* 2:62
121. Nultsch, W. 1974. In *Algal Physiology and Biochemistry,* Bot. Monogr. 10, ed. W. D. P. Stewart, pp. 864–93. Berkeley: Univ. Calif. Press. 989 pp.
122. O'Brien, T. P., Thimann, K. V. 1966. *Proc. Natl. Acad. Sci. USA* 56:888–94
123. Østlund, R. E., Willingham, M. C., Pastan, I. 1974. *Fed. Proc.* 33:1580 (Abstr.)
124. Palevitz, B. A. 1971. *Ultrastructural studies on sieve element plastids and P-proteins in the primary phloem of legumes.* PhD thesis. Univ. Wisc. Madison. 244 pp.
125. Palevitz, B. A. 1976. In *Cell Motility B,* Cold Spring Harbor Conf. Cell Proliferation, ed. R. Goldman, T. Pollard, J. Rosenbaum, Vol. 3, pp. 601–11. Cold Spring Harbor: Cold Spring Harbor Lab. 1373 pp.
126. Palevitz, B. A., Ash, J. F., Hepler, P. K. 1974. *Proc. Natl. Acad. Sci. USA* 71:363–66
127. Palevitz, B. A., Hepler, P. K. 1975. *Planta* 125:261–71
128. Palevitz, B. A., Hepler, P. K. 1975. *J. Cell Biol.* 65:29–38
129. Parthasarathy, M. V., Mühlethaler, K. 1972. *J. Ultrastruct. Res.* 38:46–62
130. Pfeffer, W. 1903. In *Physiology of Plants,* ed. A. J. Ewart p. 3. Oxford: Clarendon Press. 296 pp. 2nd ed.
131. Pickard, W. F. 1974. *Protoplasma* 82:321–39
132. Pickett-Heaps, J. D. 1967. *Aust. J. Biol. Sci.* 20:539–51
133. Pollard, T. D. 1975. In *Molecules and Movement,* ed. S. Inoué, R. E. Stephens, pp. 259–86. New York: Raven Press. 450 pp.
134. Pollard, T. D., Weihing, R. R. 1974. *C. R. Biochem.* 2:1–65
135. Porter, K. R. 1976. In *Cell Motility A,* Cold Spring Harbor Conf. Cell Proliferation, ed. R. Goldman, T. Pollard, J. Rosenbaum, Vol. 3, pp. 1–28. Cold Spring Harbor: Cold Spring Harbor Lab. 1373 pp.
136. Rebhun, L. 1959. *Biol. Bull.* 117:518–45
137. Rebhun, L. 1963. In *The Cell in Mitosis,* ed. L. Levine, pp. 67–103. New York: Academic. 274 pp.
138. Rebhun, L. 1967. *J. Gen. Physiol.* 50:223–39
139. Rebhun, L. 1972. In *Primitive Motile Systems in Cell Biology,* ed. R. D. Allen, N. Kamiya, pp. 503–25. New York: Academic. 642 pp.
140. Rebhun, L. 1975. *Soc. Gen. Physiol.* 30:233–38
141. Robidoux, J., Sandborn, E. B., Fensom, D. S., Cameron, M. L. 1973. *J. Exp. Bot.* 24:349–59
142. Rosenbush, J. P., Jacobson, G. R., Jaton, J. C. 1976. *J. Supramol. Struct.* 5:391–96
143. Sabnis, D. D., Jacobs, W. P. 1967. *J. Cell Sci.* 2:465–72
144. Sandan, T., Somura, T. 1959. *Bot. Mag.* 72:337–41
145. Sattelle, D. B., Buchan, P. F. 1976. *J. Cell Sci.* 22:633–43
146. Schmitt, F. O. 1969. *Symp. Int. Soc. Cell Biol.* 8:95–111
147. Seifriz, W. 1943. *Bot. Rev.* 9:49–123
148. Seifriz, W. 1952. In *Deformation and Flow in Biological Systems,* ed. Frey-Wyssling, pp. 3–156. Amsterdam: North Holland Publ. 552 pp.
149. Sloan, R. T., Sabnis, D. D., Hart, J. W. 1976. *Planta* 132:97–102
150. Spudich, J. A., Lin, S. 1972. *Proc. Natl. Acad. Sci. USA* 69:442–46
151. Stephens, R. E., Edds, K. T. 1976. *Phys. Rev.* 56:709–77
152. Swanson, C. A., Böhning, R. H. 1951. *Plant Phys.* 26:557–64
153. Szamier, P. M., Pollard, T. D., Fujiwara, K. J. 1975. *J. Cell Biol.* 67:424a (Abstr.)
154. Tannenbaum, J., Tannenbaum, S. W., Lo, L. W., Godman, G. C., Miranda, A. F. 1975. *Exp. Cell Res.* 91:47–56
155. Taylor, D. L., Condeelis, J. S., Moore, P. L., Allen, R. D. 1973. *J. Cell Biol.* 59:378–94
156. Taylor, E. W. 1964. In *Symposium on Biorheology,* ed. A. L. Copley, Pt. 4, pp. 175–91. New York: Wiley. 634 pp.
157. Tazawa, M. 1968. *Protoplasma* 65:207–22
158. Tazawa, M. 1975. *Bot. Mag.* 88:219–29
158a. Tazawa, M., Kikuyama, M., Shemmen, T. 1976. *Cell Struct. Funct.* 1:165–76
159. Tazawa, M., Kikuyama, M., Shimmen, T., Kiyosawa, K. 1976. *J. Cell Biol.* 70(2):125a (Abstr.)
160. Tazawa, M., Kishimoto, U. 1964. *Plant Cell Physiol.* 5:45–59
161. Tazawa, M., Kishimoto, U. 1968. *Plant Cell Physiol.* 9:361–68
162. Tazawa, M., Nagai, R. 1965. *Z. Pflanzenphysiol.* 54:333–44
163. Thaine, R. 1961. *Nature* 192:772–73

164. Thaine, R. 1969. *Nature* 222:873–75
165. Thompson, R. G., Thompson, A. D. 1973. *Can. J. Bot.* 51:933–36
166. Tilney, L. G. 1975. *J. Cell Biol.* 64:289–310
167. Tilney, L. G., Porter, K. R. 1965. *Protoplasma* 60:317–44
168. Tirosh, R., Oplatka, A., Chet, I. 1973. *FEBS Lett.* 34:40–42
169. Umrath, K. 1930. *Protoplasma* 9:576–97
170. Vorob'eva, J. A., Poglazov, B. F. 1963. *Biofizika* 8:475–77
171. Wagner, G., Haupt, W., Laux, A. 1972. *Science* 176:808–9
172. Wardlaw, I. F., Passioura, J. B., eds. 1976. *Transport and Transfer Processes in Plants.* New York: Academic. 484 pp.
173. Weatherly, P. E., Johnson, R. P. C. 1968. *Int. Rev. Cytol.* 24:149–92
174. Weber, C., Kleinig, H. 1971. *Planta* 99:179–82
175. Weihing, R. R. 1976. *Cell Biol. Handb.* I:341–57
176. Williams, N. S. 1970. *Light effects on protoplasmic streaming in Acetabularia crenulata.* MS thesis. Univ. Md., College Park. 66 pp.
177. Williamson, R. E. 1972. *Planta* 106:149–57
178. Williamson, R. E. 1974. *Nature* 248:801–2
179. Williamson, R. E. 1975. *J. Cell Sci.* 17:655–68
180. Williamson, R. E. 1976. In *Transport and Transfer Processes in Plants,* ed. I. F. Wardlaw, J. B. Passioura, pp. 51–58. New York: Academic. 484 pp.
181. Yamaha, G., Ida, M. 1933. *Protoplasma* 17:146–58
182. Yotsuyanagi, Y. 1953. *Cytologia* 18:202–17
183. Zurzycki, J. 1962. In *Handbuch der Pflanzenphysiologie,* ed. W. Ruhland, pp. 940–78. Berlin: Springer Verlag

Annual Review of Biophysics and Bioengineering
Volume 7, 1978

CONTENTS