Structural Aspects of Saltatory Particle Movement

LIONEL I. REBHUN

From the Department of Biology, Princeton University, New Jersey

ABSTRACT A variety of cells possess particles which show movements statistically different from Brownian movements. They are characterized by discontinuous jumps of 2–30 μ at velocities of 0.5–5 μ/sec or more. A detailed analysis of these saltatory, jumplike movements makes it most likely that they are caused by transmission of force to the particles by a fiber system in the cell outside of the particle itself. Work with isolated droplets of cytoplasm from algae demonstrates a set of fibers involved in both cytoplasmic streaming and saltatory motion, suggesting that both phenomena are related to the same force-generating set of fibers. Analysis of a variety of systems in which streaming and/or saltatory movement occurs reveals two types of fiber systems spatially correlated with the movement, microtubules and 50 A microfilaments. The fibers in *Nitella* (alga) are of the microfilament type. In other systems (melanocyte processes, mitotic apparatus, nerve axons) microtubules occur. A suggestion is made, based on work on cilia, that a microtubule-microfilament complex may be present in those cases in which only microtubules appear to be present, with the microfilament closely associated with or buried in the microtubule wall. If so, then microfilaments, structurally similar to smooth muscle filaments, may be a force-generating element in a very wide variety of saltatory and streaming phenomena.

In several preceding papers (1–4), we have presented a detailed description of certain types of intracellular particle movements which fall outside the scope of Brownian motion. The movements are characterized by discontinuous, stop-go periods of motion, interspersed by periods of rest; i.e. particles may suddenly move many microns in seconds where they had been essentially still before.

The neutral term saltatory, or jumplike, was applied to these movements (3, 4). The motions were shown to be present in a variety of cells and to be characteristic of a variety of particles. A detailed analysis of the behavior of the saltatory motions led to the conclusion that the mechanism giving rise to them is probably the same in all cells in which they occur and that the motive force for movement arises from a system outside the particles; i.e. the

motions are not "autonomous" (5, 6) but are induced from the particle's intracellular environment. Possible morphological entities involved were discussed, and it was concluded that a set of fibers having properties similar to those seen in isolated droplets of cytoplasm obtained from *Nitella* and *Chara* (5–14) were probably involved. It was also suggested that these fibers might be attached to the endoplasmic reticulum (4).

Some of these results will be reviewed in the light of newer findings concerning ultrastructural entities seen in systems which appear to possess particles showing motions identical to or related to saltations.

GENERAL PROPERTIES OF SALTATORY MOVEMENT

A detailed analysis of the motion of metachromatic particles in *Spisula solidissima* (clam) (1–4) eggs, yolk granules in *Pectinaria gouldii* (annelid) eggs (3, 4), melanin granules in melanocytes of early *Fundulus heteroclitus* (minnow) embryos (3), and echinochrome granules in *Arbacia punctulata* (sea urchin) eggs (3, 4, 16, 17) leads to the following generalizations concerning saltatory movements.

(1) Particles previously at rest or undergoing local Brownian movement may suddenly move along continuous trajectories, up to 30 μ in length at rates up to 5–6 μ/sec, although more usually in the 0.5–2 μ range.

(2) Neighboring particles at distances of the order of 1 μ may be suddenly separated when one particle moves while the other remains essentially in place.

(3) Conversely, several particles separated by distances of 1 μ or so may suddenly move together at the same velocity and in the same direction. The particles may form a clustered group or may move in tandem. In either case, one of a group may not move the same distance as the others, "peeling out of echelon" on the way.

(4) Maximum velocities attained and distances traversed are independent of particle size, being the same for large or small particles (on the average) and for individual particles or clusters of particles.

(5) The trajectories may be curved or straight, but the particle velocities are uniform for the major part of the journey. Often many particles will use the same trajectory and may traverse it in either direction.

(6) In many cells, only certain types of particles show the movement; e.g. in *Spisula*, metachromatic particles (and, at certain times, cortical granules); in *Pectinaria*, yolk granules; in *Arbacia*, echinochrome and metachromatic granules, etc. This selectivity is, however, not universally found. That is, in some cells, all visible particles may undergo saltatory movements (19).

(7) The number of particles undergoing saltation, the distance moved, and the velocity attained may vary depending on the stage of the cell cycle observed.

(8) In general, all three parameters mentioned in the preceding paragraph increase during prophase of mitosis and continue at an elevated level through much of mitosis (however, see below).

(9) In many cells, saltations are randomly oriented. However, in pseudopods of migrating pigment cells in *Fundulus* embryos, in *Arbacia* eggs after fertilization when the echinochrome granules migrate to the cortex, in extended processes of amphibian fibroblasts in tissue culture, in HeLa cells (19), and especially in mitosis, the movements become polarized. In the last case, the motions become radial to the centrosome with the preponderant movement toward that body, although saltations radially away at lower frequency of occurrence and shorter distance traveled also occur (1, 3, 4, 20, 21).

A detailed illustration of some of these points can be seen in particle movements in newt cells in tissue culture. The following discussion and data are taken from a senior honors thesis done in our laboratory by Mr. Jack Chidester [see also Taylor (21)]. Newt lung cells were cultured in Sykes-Moore chambers in Rothstein's medium. Time lapse movies were taken on 16 mm film at a rate of one exposure each 2 sec at room temperature, about 22°C. The film was analyzed frame by frame using an L and W photoanalyzer projector (Milwaukee, Wisc.). Newt cells grow extremely flat under our conditions, and essentially all particles can be seen in a given cell at one focal level with a 40 × lens. Table I contains an analysis of 40 particles seen to move from early to late prophase. The mean distance traveled is about 4.3 μ, with an average speed of about 0.36 μ/sec. The average distance traveled by 31 particles in two cells in interphase was somewhat higher, 5.45 μ, with average velocity of 0.41 μ/sec. From early metaphase to anaphase, a total of 65 particles were seen to move, with average distance traveled of 6.72 μ and an average velocity of 0.63 μ/sec. Table II includes only data from early to mid-metaphase. It should be noted that individual velocities of particles during metaphase may become more than twice those seen in prophase or interphase although the averages are not quite that high. Also, particle direction cannot be listed easily in the tables, but, in general, directions are radial to the asters in metaphase and not specifically oriented prior to nuclear envelope disappearance.

In this material, but not in marine eggs, saltations tend to subside during anaphase. In the film sequence whose partial analysis resides in Tables I and II, only four saltations were seen during anaphase and these all were in particles which moved parallel to the spindle axis, starting in the *interzone* between separating chromosomes and moving toward the poles.

In general, for this material, a detailed analysis reveals all nine characteristics initially listed except for the decrease in the number of particles moving in anaphase and the lower average velocity of movements in these cells compared to egg cells. These data are not easy to obtain since saltations must be recognized in the film, observed at normal speed, replayed and plotted

TABLE I

NEWT MITOSIS

Series 1—Early Prophase to Late Prophase

(1044 frames, 34 min, 48 sec)

Particle No.	Total displacement	Time	Average speed of saltation
	μ	*sec*	μ/*sec*
1	1.70	6	0.28
2	3.00	6	0.50
3	3.17	8	0.40
4	7.52	20	0.38
5	4.06	8	0.51
6	13.98	42	0.33
7	3.20	16	0.20
8	2.65	8	0.33
9	3.75	14	0.27
10	8.19	26	0.32
11	6.06	22	0.28
12	9.08	26	0.35
13	7.96	22	0.36
14	2.54	8	0.32
15	1.64	6	0.27
16	8.33	20	0.42
17	2.02	8	0.25
18	4.32	14	0.31
19	4.04	8	0.51
20	3.86	10	0.39
21	3.69	8	0.46
22	2.22	6	0.37
23	1.94	10	0.20
24	2.28	4	0.57
25	3.14	6	0.52
26	6.46	16	0.40
27	3.17	8	0.40
28	3.14	8	0.39
29	2.34	8	0.29
30	2.25	8	0.28
31	3.14	8	0.39
32	3.97	8	0.50
33	4.21	12	0.35
34	2.34	8	0.29
35	2.34	6	0.39
36	8.07	16	0.50
37	1.64	6	0.27
38	1.99	12	0.16
39	4.61	16	0.29
40	6.95	26	0.27
Mean	4.28		0.36
SD	2.67		0.097

TABLE II

NEWT MITOSIS

Series 3—Early to Mid-metaphase

(1400 frames, 46 min, 12 sec)

Particle No.	Total displacement	Time	Average speed of saltation
	μ	*sec*	μ/*sec*
1	4.44	8	0.56
2	3.20	6	0.53
3	5.70	12	0.48
4	5.42	10	0.54
5	9.37	10	0.94
6	8.91	14	0.64
7	2.31	4	0.58
8	5.91	12	0.49
9	8.76	16	0.55
10	4.84	6	0.81
11	2.65	6	0.44
12	6.19	12	0.52
13	6.75	28	0.24
14	7.06	14	0.50
15	4.90	10	0.49
16	4.58	6	0.76
17	5.13	12	0.43
18	6.08	10	0.61
19	5.62	10	0.56
20	4.76	10	0.48
21	10.75	16	0.67
22	4.27	8	0.53
23	14.33	22	0.65
24	15.83	20	0.79
25	6.63	6	1.11
26	2.04	4	0.51
27	11.53	18	0.64
28	8.79	8	1.10
29	2.31	4	0.58
30	7.38	10	0.74
31	5.80	12	0.48
32	4.96	4	1.24
33	7.93	10	0.79
34	10.21	22	0.46
35	3.95	16	0.25
36	3.32	22	0.15
37	5.51	12	0.92
Mean	6.44		0.62
SD	3.16		0.231

frame by frame, and then analyzed, but more data are being collected. In addition to the systems described above, Watters (22) has given data on movements similar to these in axopods of Heliozoa, and Burdwood (23) has considerable data on such movements in nerve axons. Few other cells have been analyzed in detail with saltatory movement in mind. However, from the descriptions given it appears certain that the same phenomenon has been observed for naturally occurring heme granules in *Urechis* eggs (20), microkinetospheres in HeLa cells [a description of saltatory movement in HeLa cells was provided by Freed (19)], various inclusions in protozoa, and especially introduced carmine particles in *Stentor* (6, 24). This material is more fully reviewed elsewhere (3, 4), where other cases which result in astral localization of particles seen by classical fixation techniques are discussed as probable examples of the same phenomenon, as are the centrosomal localizations of microbodies and dense bodies seen in the electron microscope studies of regenerating rat liver by Dougherty (18).

Thus, these movements are ubiquitous if not universal and compel attention. It should be noted that one reason that they probably are not more universally reported may be the much slower filming speeds used in most studies where chromosomal events or pinocytosis is being investigated. In these cases, movements with velocities of the order of 0.5–1 μ/sec generally are not recorded as continuous particle trajectories. Instead, close observation often reveals particles suddenly disappearing or appearing in different parts of the cells, since particles may traverse their entire path between frames at slow filming speeds.

MOVED OR SELF-MOVING?

A detailed discussion of whether the motive force for movement originates within or without the particle has been given (3, 4, 25). Basically, if a force arising within the particles gives rise to movement it must be by some jet propulsion process, a swimming process due to particle surface movements or due to influx and ejection of material. All these processes are difficult to reconcile with property 3 above, since we would be required to imagine that they start and stop in independent particles simultaneously and are all oriented precisely in the same direction. Saltatory movements exhibited by inert particles, such as heme granules in *Urechis* (20) eggs, carmine particles in *Stentor* (6, 23), and carbon particles in HeLa cells, also suggest that these movements do not originate in the particles themselves. A more thorough analysis on physicochemical grounds was given by Taylor (25) in rejecting this type of mechanism for movement.

If the particles are moved, rather than being self-moved, it is unlikely that general field forces existing throughout the cell are involved, since neighboring particles can move in different directions, the motions are often randomly

oriented, and particles may reverse their direction of movement (1, 3, 4, 19). Cogent arguments against the involvement of electrophoretic models or of general hydrodynamic forces are given on an observation level by Rebhun (1, 4) and on a physical level by Taylor (25).

In general, one appears to be left with models in which particles are moved by forces transmitted from outside the particles themselves, in which the force is developed locally rather than globally in the cell, and in which the force developed appears to be uniformly applied during the whole excursion (see property 5). It seems most likely that a dispersed set of fibers or surfaces in the cytoplasm, oriented by mitosis and capable of delivering the required force, would fit the qualifications well [a more extended discussion has been given (3, 4)].

RELATION TO CYTOPLASMIC STREAMING

A number of particle movements in plants fit the criteria we have laid down for saltatory movement except that the motions rarely are interspersed with periods of rest and generally are of higher velocity (26, 27). One of the most relevant to our discussion is the type of particle motion ocurring in isolated droplets of cytoplasm obtained from *Chara* or *Nitella*. These droplets are obtained either by squeezing cytoplasm from an internodal cell whose end has been amputated or, more elegantly, by allowing cytoplasm to flow from a cut cell into an artificial "cell sap," maintaining slight negative pressure on the cell to prevent it from collapsing (5, 14, 28).

Observation of these droplets with the bright-field microscope reveals many particles in an active motion which appears to be Brownian, although this has not been determined statistically (5). However, a number of particles may suddenly depart from this movement, undergo motions with extended trajectories, during which time the Brownian motion is not apparent, then suddenly return to Brownian motion again. Moreover, the velocity of the particles is generally uniform during the motion, and many particles may use the same "track" [see extended discussion by Kamiya (5)]. In fact, other than velocity, most of the characteristics of these movements are similar if not identical to properties 1 through 7 of the first section of this paper. We feel, therefore, that we may assume saltatory particle movements in these isolated droplets to result from mechanisms similar to those occurring in other cells, until we are forced to abandon this position by contrary evidence, if such ever materializes. These observations have been pushed considerably farther, primarily through the efforts of Jarosch (7–9, 28, 29) and of Kamiya and his group (10, 12, 14). Briefly, Jarosch showed, using dark-field microscopy, that the droplets possessed a set of fine fibrils which moved through the cytoplasm at high velocity, the forward motion being accompanied by a countermoving cytoplasmic stream. Occasionally such fibrils became attached to the droplet

wall and thus were immobilized. The countercurrent stream continued to move. If saltating particles were observed, it became clear that the disappearance of Brownian motion, embarkation on a uniform velocity journey, and termination into random movement were all dependent upon capture of the particles by the countermoving stream generated by a moving (or stationary) fibril. The rotation of nuclei or individual chloroplasts, and the snakelike movements of chains of chloroplasts, appeared to be coupled to the activity of the fibrils, which could form reversible associations with these larger inclusions.

A final, and remarkable, bit of behavior engaged in by these fibrils was that of self-aggregation. The usual result of this process was formation of polygonal structures with sharp corners which showed rather bizarre movements. Either a polygon as a whole rotated as a stiff structure, or else deformation waves propagated around the polygon (5, 9, 14).

These remarkable fibrils were thought by Jarosch to be responsible for streaming in *Nitella* when arranged in a more organized way on the inner chloroplast layer. Recently, Kamitsubo (10, 11) showed that fibers can be seen in intact but centrifuged *Nitella* cells, which are arranged parallel to the streaming direction and in such a way that streaming takes place along their surfaces. He has also succeeded in demonstrating that rotating polygons such as those described above in droplets can be "folded off" such fibers. This is about as direct evidence as I know of that the moving fibrils are related to and derived from stationary ones most likely associated with the force generation system in cytoplasmic streaming. We shall examine the structure of such fibrils later.

Thus, observation and theoretical (though negative) arguments strongly point to an underlying fiber system as generating the motive force for saltatory movement. Direct observation verifies the participation of visible fibers in such movements in *Nitella.*

PULLING OR SLIDING MODELS FOR SALTATORY MOVEMENT

The results in *Nitella* discussed above, added to a considerable amount of other material, led to the conclusion that saltatory particle movements and cytoplasmic streaming in this plant originate from forces developed in association with fibrils. The forces are somehow exerted on a passive cytoplasm causing it, and particles in it, to move parallel to the fibrils. We may classify this as a sliding model for streaming and saltation.

It may be fairly asked, however, whether the similarities between saltatory movements in animal and plant cells are illusory and if sliding models are generally applicable. We cannot directly demonstrate this, and indeed, Parpart (16, 17) in analyzing echinochrome granule movement in *Arbacia,*

always assumed that the particles moved because they were attached to contractile strings or fibrils. This is a most reasonable picture for those phenomena in which only a restricted class of particles move, such as echinochrome granules in *Arbacia*, or metachromatic granules in *Spisula* eggs (1, 4). This is, indeed, the major observational stumbling block to universal acceptance of a sliding model for all saltatory movements. A contractile model for saltatory movements was also suggested by du Praw (30).

Thus, we must consider two possible mechanisms for saltation: those that arise from sliding [or shearing (31)] forces and those that arise from contraction of fibrils (the latter being primarily a hypothetical possibility).

In discussing this problem, we may be better guided if we gain some insight into the ultrastructure of systems which show cytoplasmic streaming.

ULTRASTRUCTURAL STUDIES

A variety of streaming systems have now been investigated on the ultrastructural level, and in general two types of fibrillar material have been seen in such systems. In one type, the primary structure seen is the microtubule. The best example of this in which known streaming and saltatory movements (22) occur is in the axopods of heliozoans. The axial rod is composed of microtubules arranged in a double interlocking spiral. Tilney has shown the axial rod to be sensitive to colchicine, pressure, etc. (32), and there seems little doubt that axopodal streaming is dependent upon its continued existence.

Another prime example of the presence of microtubules in the region of saltatory movement is the mitotic apparatus. Here, saltation can occur over the spindle surface, traversing the distance between poles repeatedly during metaphase (1–4). The astral ingathering of particles (property 9 and above) is also clearly a case where microtubules, in the astral rays, parallel the direction of saltatory movement.

A case of lesser certainty may be in the Foraminifera (33, 31), where Burdwood (personal communication) has demonstrated bundles of microtubules occupying the core of reticulopodia. Saltatory particle movement in nerve axons (24) may very well be correlated with the presence of microtubules known to occur there. Finally, Bickle et al. (34) have shown that extended processes of *Fundulus* melanocytes contain microtubules, and that melanin granules can migrate up and down the processes and appear to possess some properties of "independence" of movement, making it likely to be similar to movements described here. We have described saltatory movement in this same system at an earlier embryonic stage (3, 4).

In the above cases, a correlation exists or is likely between the occurrence of saltatory movements and the presence of microtubules oriented in the proper direction to be, at least, tracks for directing these movements.

Another type of fibrillar system has been reported in noncellular slime

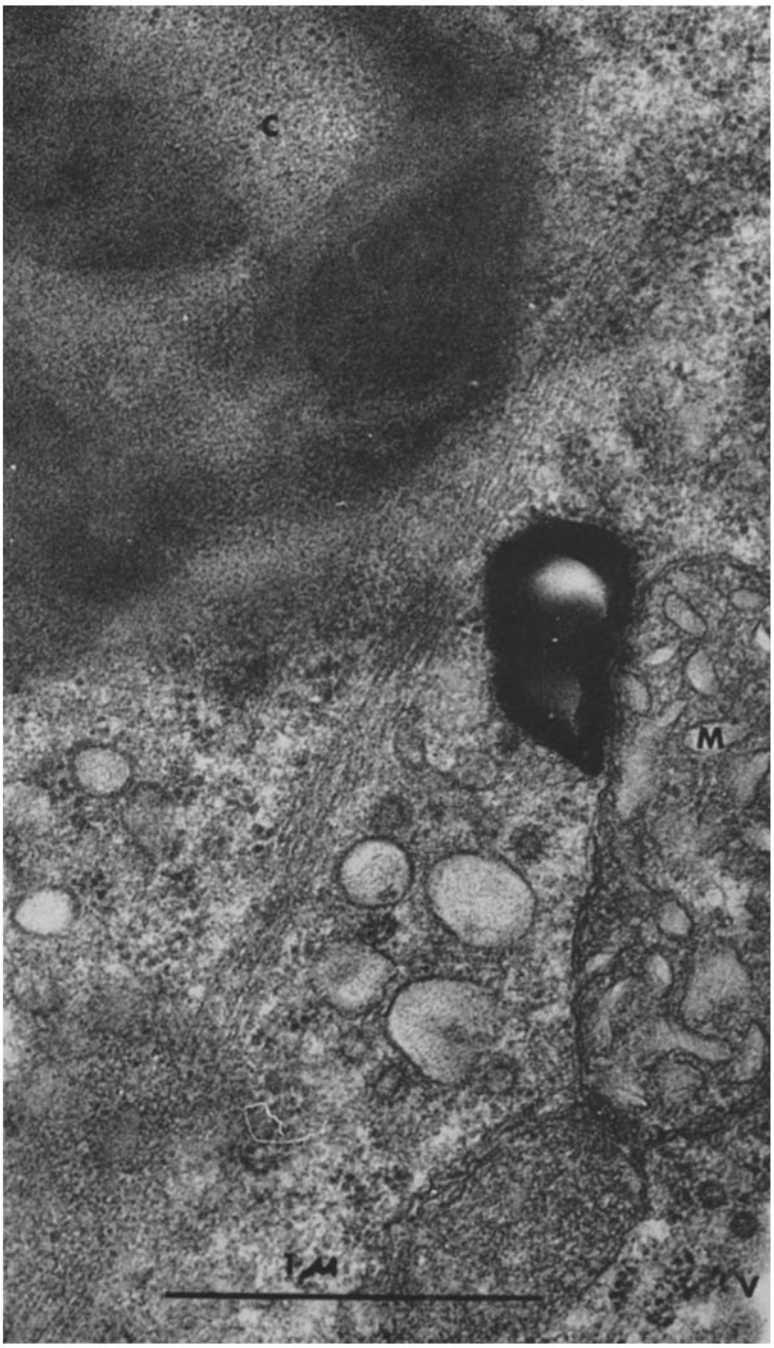

FIGURE 1

molds and correlated with generation of the motive force for streaming (35, 36). The totality of evidence in this system indicates that flow is due to pressure created by contraction of the fibrillar system. The fibrils consist of bundles of microfilaments about 60–68 A in diameter (35, 36).

Recently, Kamiya (12) has shown that glycerinated slime mold will undergo contraction on the addition of ATP. The contraction occurs in such a manner as to suggest its direct involvement in force generation for streaming. Nagai and Kamiya (37) have shown that the bundles of microfilaments survive glycerination, and, indeed, undergo an interesting lateral aggregation on addition of ATP.

It would appear, therefore, that streaming in this system is due not to the development of sliding forces but to the exertion of pressure resulting from contraction in a fibrillar system reminiscent of smooth muscle.

A recently analyzed organism in which contraction also occurs is that of *Difflugia*, the armored ameba. Here, Wohlman (38) has shown the existence of bundles of microfilaments of dimensions similar to those of slime mold and oriented in such a way as to probably represent the oriented birefringent material which develops during contraction of the pseudopod.

A final case may be that of the contractile system of ciliates and other protozoa, where Yagai and Shigenaka (39) and Nagai and Rebhun (40) have suggested the possibility that the M bands (bundles of microfilaments), and not the Km fibers (41) (microtubules), may be the mechanical basis of contraction.

Thus, it would appear from this incomplete inventory that a tentative hypothesis could be suggested—namely, that wherever saltation is the phenomenon observed, microtubules are involved, and wherever contractility is found as the basis for cytoplasmic streaming, microfilaments are involved. This neat summary, is, unfortunately, not able to survive further scrutiny. Indeed, in the one case where saltatory particle movements *and* cytoplasmic streaming have been directly observed to be related to activity of fibrils on the light microscope level, the latter have been shown to consist of microfilaments, not microtubules (40). Thus, Nagai and I have shown in *Nitella* that bundles of 50–60 A microfilaments (40) lie oriented in such a way as to leave no doubt that they correlate well with Kamitsubo's (10, 11) fibers seen with the light microscope (Figs. 1 and 2). In addition, they are found on the chloroplast surface at the boundary, where much work has shown the motive force for streaming to be developed (5). Microtubules are seen, but only

---

FIGURE 1. A longitudinal section of a microfilament bundle in a *Nitella* internode cell, oriented in the direction of cytoplasmic streaming. C = chloroplast, M = mitochondrion, V = central vacuole. × 61,000.

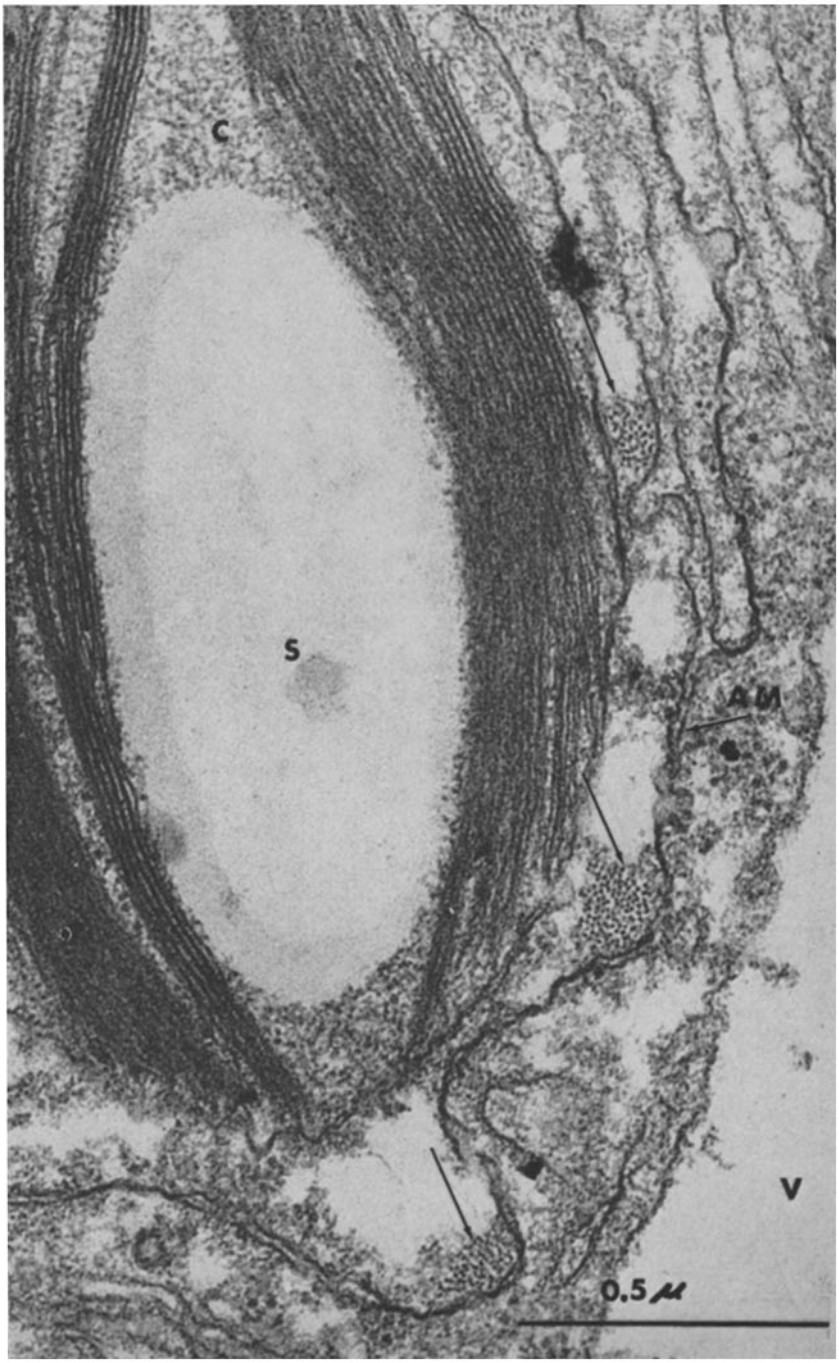

FIGURE 2

below the chloroplasts at the plasma membrane, at least 1 μ away from the moving stream.

Finally, in very preliminary work just prior to her return to Japan, Dr. Nagai obtained some sections of isolated droplets in which bundles of microfilaments could be observed (Fig. 3), with a morphology similar to that seen in the intact cell. This suggests the identity of microstructure between droplet filaments and polygons with filaments from intact cells, an idea made very reasonable by Kamitsubo's observations of the derivation of motile filaments and rotating polygons from stationary fibers in intact *Nitella* internodes.

This work has recently been verified in a different system, oat coleoptile, where O'Brien and Thimann (42) have shown bundles of microfilaments oriented parallel to the streaming direction and microtubules present but not correlated in orientation with streaming.

Thus, nature has not seen fit to present us with an easy, neat solution to our problem. Another puzzle not readily solved is seen in the next section.

COLCHICINE AND SALTATORY MOVEMENTS

In assessing the type of structure involved in saltatory motion in animal cells, it would be convenient to be able to destroy selectively one or the other of the types of structures discussed and to view its effects on saltation. Since microtubules are broken down by colchicine, e.g. in heliozoan axostyles (32) and in interphase cells (15), as well as in the mitotic apparatus, we may hope to use this agent if it is selective. According to P. Green (personal communication), cellulose microfibril orientation is disturbed in cell wall growth in *Nitella*, but streaming is unaffected by colchicine. This indicates that colchicine enters the cell and destroys the peripheral microtubules but does not affect the microfilaments. We may, therefore, tentatively assume that colchicine is a selective agent with desired properties.

Freed (19) has shown that saltatory movements in interphase are prevented if HeLa cells are treated with colchicine. Given the demonstration by Robbins and Gonatas (15) that microtubules are lost after such treatment, Freed (19) assumed that the loss of microtubules and the loss of saltatory movement are directly correlated.

Armed with these results, we recently used colchicine on unfertilized *Arbacia* eggs to see if saltatory movement of the echinochrome granules was inhibited by the drug. Much to our surprise, doses of 10^{-3} M colchicine (10^{-5}

FIGURE 2. Cross-sections of filament bundles (arrows) in a *Nitella* internode cell. The section is perpendicular to the axis of the cell, which is almost perpendicular to the streaming direction (see reference 40). S = starch granule in the chloroplast, AM = agrangular membrane often associated with microfilament bundles. Other letters as in Fig. 1. × 80,000.

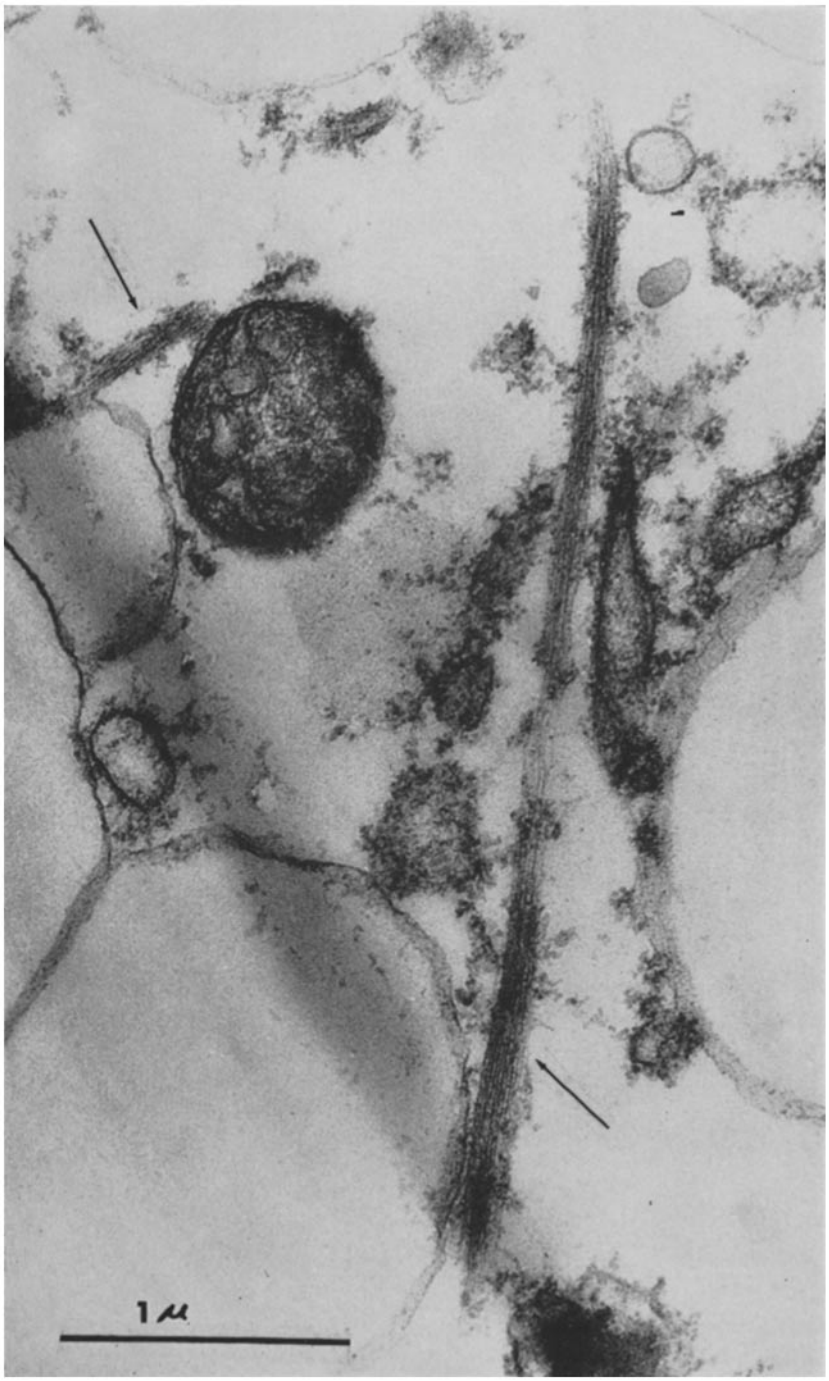

FIGURE 3

M prevents the mitotic apparatus from forming) had no visible effect on such movements as seen in time lapse films. Although no statistical data has yet been obtained to see if slight effects are present, in general the frequency, velocity, and trajectory lengths appear to be very similar in control and treated eggs. But does colchicine enter the unfertilized egg? To answer this we verified a result of Cornman and Cornman (43) that eggs, treated before fertilization and then washed free of colchicine (the Cornmans actually used podophyllin) prior to fertilization, still showed only a few irregular cleavages.

Thus, *Arbacia* eggs were treated with 10^{-3} M colchicine for about ½ hr, washed free of colchicine with three 100:1 washes of sea water, and fertilized. They were compared with eggs left in colchicine. No difference could be seen; neither showed significant cleavage by the time untreated controls had cleaved normally. However, pigment migration to the cortex, an event occurring by directed saltatory movements within 10 min after fertilization, occurred normally.

Thus, there can be little doubt that colchicine does enter the *Arbacia* egg but that it has little or no effect on saltatory movement at doses 100 times higher than those sufficient to inhibit mitotic apparatus and, thus, microtubule formation. Does this mean that microtubules are *not* involved in saltatory movement in *Arbacia*? This is a conclusion difficult to avoid. However, for complicated reasons we prefer not to cross this bridge completely at this time.

CONCLUSION

Is there some way in which we can reconcile microtubule or microfilament hypotheses? One way is the suggestion of O'Brien and Thimann (42) that these structures are alternate states of the same material, organized under certain conditions as microfilaments, when motive force generation is paramount, and organized under others as microtubules, when structural rigidity is necessary.

We would like to suggest an alternative view based on the exemplary work of Gibbons on cilia (31). In cilia, an ATPase, presumably the force-generating species, is reversibly associated with microtubules, the presumed rigid elements. Thus we wish to suggest that a separate force-generating species is or can be associated with microtubules wherever they are involved in generation of motive force. If so, however, where are they? No visual evidence is seen for their existence.

To get some hold on the problem, we refer to a calculation due to Wolpert

---

FIGURE 3. Microfilament bundles (arrows) from isolated droplets of *Nitella* cytoplasm. The two bundles seen here may possibly be part of a polygon (see the text). × 38,000.

(44). He asked what the average force is which is exerted between two moving filaments in the sliding filament theory of striated muscle contraction. Using known values of force development in muscle as a function of muscle diameter and number of thick-thin filament pairs, he obtained the value of 3×10^{-5} dyne. Using viscosity theory, he calculated the force needed to move a 1 μ particle in saltation at a rate of 5 μ/sec in a medium of viscosity 10 centipoise. The value is 10^{-5} dyne.

This calculation is not meant as a convincing demonstration of the similarity between the forces developed in muscular contraction and in saltatory movement. It merely demonstrates that, if the structures involved in saltation can develop forces anything near those developed in filament interaction in striated muscle, then not many filaments are needed to account for saltatory movement. Indeed, if each microtubule, e.g. in heliozoan axostyles, is associated with one 50 A microfilament, enough motive force might be developed to account for the observed saltatory movement. It would not be easy to locate such a structure in cross-sections of microtubules if it were closely applied to the microtubule wall or if it lay in a groove between subunits of the microtubule.

The motivation for the preceding speculations has been to see if a unified overview can be obtained for phenomena ostensively so widely divergent as as cytoplasmic streaming, nerve axoplasmic transport, and mitosis. We hope that the plausibility of this view is clear. Whether it is actually true will have to depend on the results of experiments rather than discussion, with which this paper is, perhaps, overburdened.

I especially wish to thank Mrs. Greta Sander and Dr. Reiko Nagai for their suggestions and help in many aspects of this work.

These investigations have been supported by grants from the National Science Foundation, the National Institutes of Health, and the American Cancer Society.

REFERENCES

1. REBHUN, L. I. 1959. *Biol. Bull.* **117**:518.
2. REBHUN, L. I. 1960. *Ann. N.Y. Acad. Sci.* **90**:357.
3. REBHUN, L. I. 1963. *In* The Cell in Mitosis. L. Levine, editor. Academic Press, Inc., New York. 67.
4. REBHUN, L. I. 1964. *In* Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 503.
5. KAMIYA, N. 1959. *Protoplasmatologia.* **8**:13a.
6. SEIFRITZ, W. 1952. *In* Deformation and Flow in Biological Systems. A. Frey-Wyssling, editor. Amsterdam. 3.
7. JAROSCH, R. 1958. *Protoplasma.* **50**:93.
8. JAROSCH, R. 1960. *Phyton (Buenos Aires).* **15**:43.
9. JAROSCH, R. 1964. *In* Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 599.
10. KAMITSUBO, E. 1966. *Proc. Japan Acad.* **42**:507.

11. KAMITSUBO, E. 1966. *Proc. Japan Acad.* **42**:640.
12. KAMIYA, N., and K. KURODA. 1965. *Proc. Japan Acad.* **41**:837.
13. KITCHING, J. A. 1964. *In* Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 445.
14. KURODA, K. 1964. *In* Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 31.
15. ROBBINS, E., and N. GONATAS. 1964. *J. Histochem. Cytochem.* **12**:704.
16. PARPART, A. K. 1953. *Biol. Bull.* **105**:368.
17. PARPART, A. K. 1964. *In* Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 471.
18. DOUGHERTY, W. D. 1963. Doctorate Thesis. Princeton University.
19. FREED, J. J. 1965. *J. Cell Biol.* **27**:29A (Abstr.).
20. TAYLOR, C. 1931. *Physiol. Zool.* **4**:423.
21. TAYLOR, E. 1957. Doctorate Thesis. University of Chicago.
22. WATTERS, C. 1965. Doctorate Thesis. Princeton University.
23. ANDREWS, E. A. 1955. *Biol. Bull.* **114**:113.
24. BURDWOOD, W. 1965. *J. Cell Biol.* **27**:115A. (Abstr.)
25. TAYLOR, E. 1964. *Proc. Intern. Congr. Rheol., 4th, Providence, R.I.* 175.
26. HONDA, S. I., T. HONGLADAROM, and S. G. WILDMAN. 1964. *In* Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 485.
27. MAHLBERG, P. G. 1964. *In* Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 43.
28. JAROSCH, R. 1956. *Phyton (Buenos Aires).* **6**:87.
29. JAROSCH, R. 1957. *Biochim. Biophys. Acta.* **25**:204.
30. DU PRAW, E. J. 1965. *Develop. Biol.* **12**:53.
31. JAHN, T., and R. A. RINALDI. 1959. *Biol. Bull.* **117**:100.
32. TILNEY, L. G. 1965. *J. Cell Biol.* **27**:107A. (Abstr.)
33. ALLEN, R. D. 1964. *In* Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 407.
34. BICKLE, D., L. G. TILNEY, and K. R. PORTER. 1966. *Protoplasma.* **61**:322.
35. WOHLFARTH-BOTTERMAN, K. 1964. *In* Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 79.
36. WOHLFARTH-BOTTERMAN, K. 1964. *Intern. Rev. Cytol.* **16**:61.
37. NAGAI, R., and N. KAMIYA. 1966. *Proc. Japan Acad.* In press.
38. WOHLMAN, A. 1965. Doctorate Thesis. Princeton University.
39. YAGAI, R., and Y. SHIGENAKA. 1963. *J. Protozool.* **10**:364.
40. NAGAI, R., and L. I. REBHUN. 1966. *J. Ultrastruct. Res.* **14**:571.
41. RANDALL, J. T., and S. FITTON-JACKSON. 1958. *J. Biophys. Biochem. Cytol.* **4**:807.
42. O'BRIEN, T. R., and K. V. THIMANN. 1966. *Proc. Natl. Acad. Sci. U. S.* **56**:888.
43. CORNMAN, I., and M. E. CORNMAN. 1951. *Ann. N.Y. Acad. Sci.* **51**:1443.
44. WOLPERT, L. 1965. *Symp. Soc. Gen. Microbiol.* **15**:270.

NOTE A Discussion of the Session on Contractile Processes in Nonmuscular Systems begins on page 288.