*INTRACELLULAR FIBERS IN OAT COLEOPTILE CELLS AND THEIR POSSIBLE SIGNIFICANCE IN CYTOPLASMIC STREAMING**

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During the course of an intensive investigation of the histology and fine structure of the *Avena* coleoptile,[1–4] we have had the opportunity to examine the varied cell types of the organ, fixed in a number of ways. In tissues fixed initially either in acrolein or glutaraldehyde, and subsequently postfixed in osmium tetroxide, intracellular fibers have been seen in the cells of the inner and outer epidermis, and in the vascular and extrafascicular parenchyma. Similar fibers have been reported in amebae and slime molds[5] and most recently in *Nitella*,[6] and fibrous structures appear, of course, in sieve tubes after certain fixation procedures.[7] However, to our knowledge, fine intracellular fibers have not been described previously in the parenchyma cells of higher plants.

Materials and Methods.—Coleoptiles of *Avena sativa* var. *"Victory"* were grown to a length of 25 mm under the conditions described previously; the procedures of specimen preparation for electron microscopy have been given in detail.[3] Fibers have been identified in cells from 25-mm coleoptiles fixed in 10 per cent acrolein at 0°C for 12–16 hr in tap water (or at room temperature for 5 hr), or in 5 per cent glutaraldehyde at 0°C (in 0.025 *M* sodium phosphate buffer at pH 6.8 for 12–16 hr). All tissues were washed briefly in buffer and postfixed in 2 per cent osmium tetroxide at 0°C for 12–16 hr. The cells of these mature coleoptiles were found to be badly preserved in glutaraldehyde at room temperature and it is not possible to say whether the fibers are preserved by that fixation.

Observations.—The fibers are 0.1–0.2 μ wide and of indefinite length; the one shown in Figure 1 extended for at least 10 μ in the section. They occur both in transvacuolar strands and in the parietal cytoplasm (Figs. 2, 4, and 7). Unlike microtubules, they do not seem to occur in the extreme cortex of the cell, but tend rather to lie about halfway between the plasmalemma and the tonoplast. Ribosomes seem to be excluded from the matrix of the fibers (Fig. 6), but mitochondria are commonly associated with them and indeed seem often to be in contact with them (Figs. 1 and 7).

The apparent substructure of the fibers varies with the fixation procedure. In tissue fixed initially in glutaraldehyde, the fibers consist mainly of a highly ordered array of microfilaments, each 50 to 70 Å wide and of indefinite length. Here and there along the length of the fibers, the orderliness of the array is not maintained (Fig. 4). These microfilaments are closely similar to those described in *Nitella*.[6] In tissues fixed initially in acrolein, on the other hand, the fibers show much less filamentous substructure. Instead, they have a feltlike appearance, particularly when fixed in acrolein at room temperature (Fig. 5). It is impossible to decide from the present evidence which of these images corresponds more closely to the *in vivo* structure of the fiber. The clarity of structure of the fibers in slime mold plasmodium is similarly dependent on fixation in glutaraldehyde.[8] Nagai and Rebhun[6] were able to show the appearance of the *Nitella* fibers in cross section but

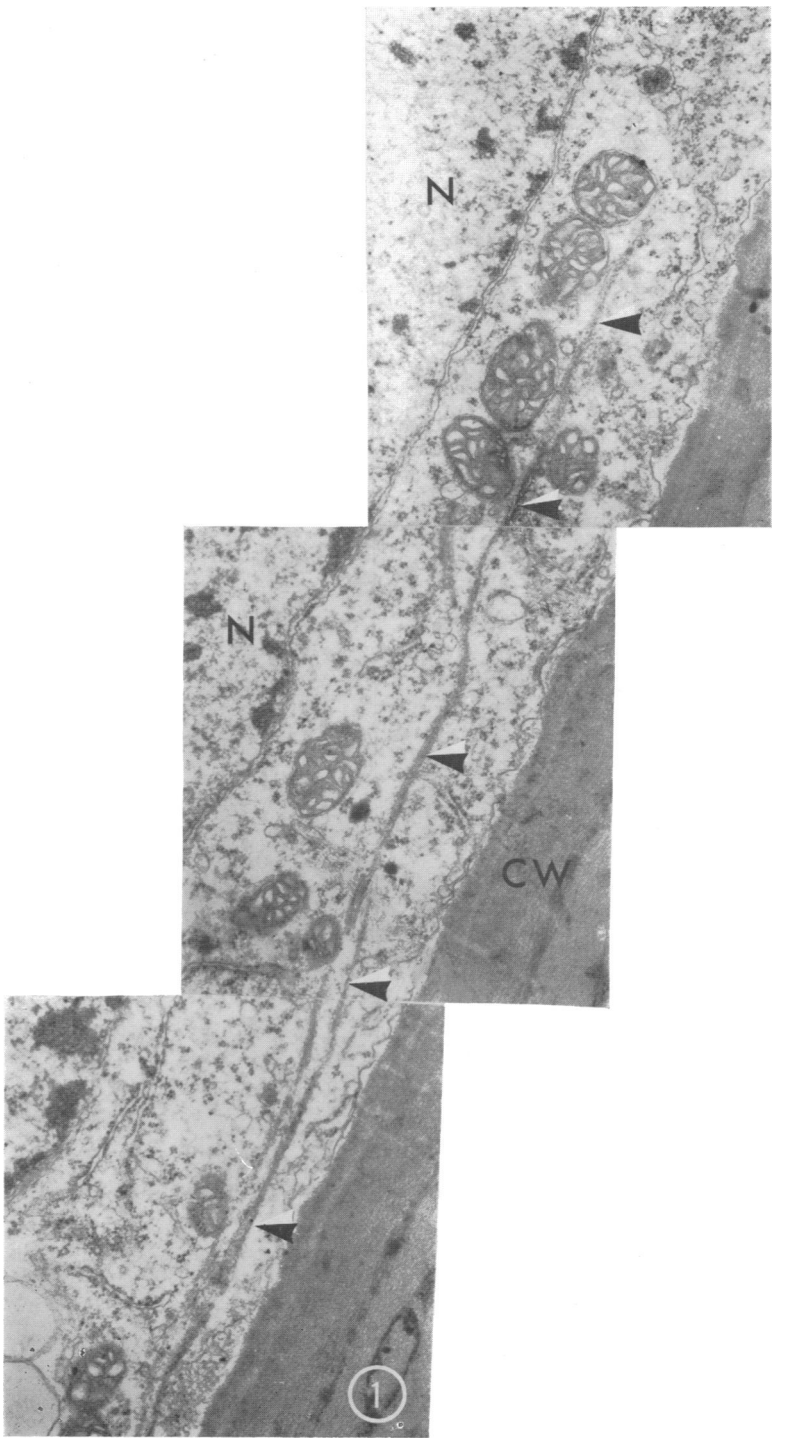

FIG. 1.—Montage to show intracellular fibers (*arrows*) in a xylem parenchyma cell. Acrolein/osmium fixation. *N*, Nucleus; *CW*, cell wall; ×19,000.

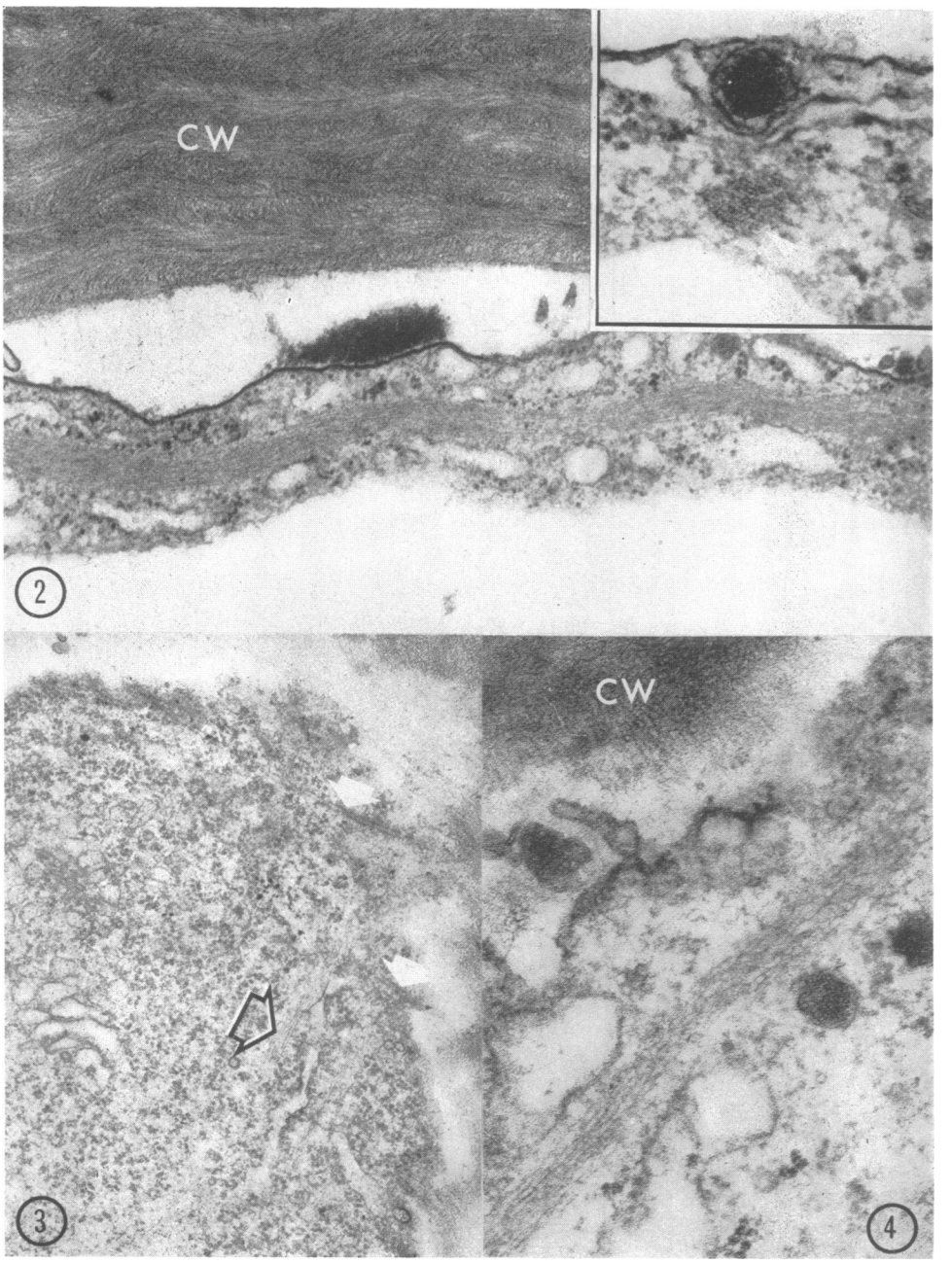

FIG. 2.—Fiber in cell of the outer epidermis, longitudinal section, ×34,860. *Inset:* probable cross section of a fiber (*arrow*), ×51,200. Both, glutaraldehyde/osmium fixation.

FIG. 3.—Cell of the vascular parenchyma, showing cortical microtubules (*solid white arrows*) and a fiber arranged at right angles to the direction of the microtubules (*open arrow*). Glutaraldehyde/osmium, ×28,782.

FIG. 4.—Part of a fiber in an outer epidermal cell showing the microfilaments in both strictly and partially ordered array. Glutaraldehyde/osmium, ×75,932.

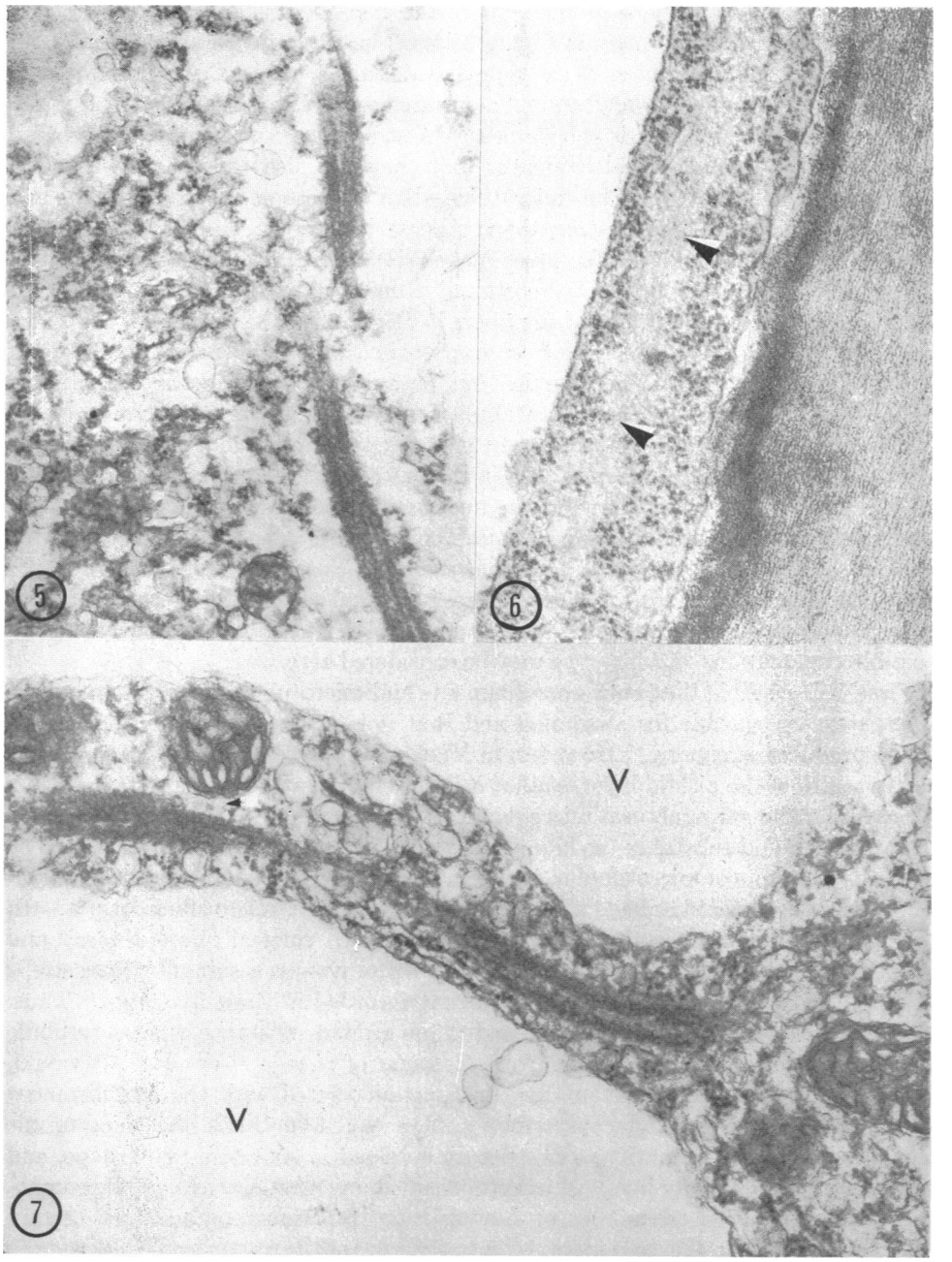

FIG. 5.—Parenchyma cell after fixation in acrolein at room temperature, showing parts of two fibers, ×29,520.

FIG. 6.—Cell of the inner epidermis, showing a fiber at the arrows. Note the complete exclusion of the ribosomes from the matrix of the fiber. Glutaraldehyde/osmium fixation, ×39,688.

FIG. 7.—A transvacuolar strand of cytoplasm in a parenchyma cell fixed in acrolein at 0°C. Note the close association of the mitochondria with the strand (*arrow*). *V*, vacuole, ×34,860.

it has proved to be less easy to do so in the coleoptile cells, since it is quite difficult to distinguish them from other components of the ground substance; however, it is likely that the structure shown in Figure 2 (*inset*) is a fiber in cross section.

Discussion.—Microtubules, of the type first described in plant cells by Ledbetter and Porter,[9, 10] were considered to be "associated with the development and maintenance of highly asymmetric cell shapes." A strong case can be made,[10–13] based admittedly on circumstantial evidence, that they are also concerned with cytoplasmic movement.[12–14] The microtubule-like structures of the spindle and axonemes of cilia and flagella strengthen this case. However, the correlation is not perfect, for movement may take place in association with a fibrous component of the ground substance which is *not* organized as microtubules. The successive differentiation and disappearance of the fibers in *Physarum*, with change in the direction of streaming, as well as their orientation in the cytoplasmic strands, speak strongly for their causal function in that organism.[8] Furthermore, Nagai and Rebhun[6] could not detect any microtubules in that region where the motive force for streaming in *Nitella* is believed to be generated, although long fibers composed of microfilaments similar to those seen in the oat coleoptile are present there. We have considered the possibility that the bundles of microfilaments might have been derived from microtubules during fixation, but this is rendered unlikely by the presence of *both* microtubules and microfilaments in closely adjacent regions of the same cell (Fig. 3, cf. ref. 6). A parallel case in animal cells is the presence of both microtubules and microfilaments in melanocytes.[15] There are obviously several possible explanations, of which two may be considered here.

First, it is possible that both microfilaments and microtubules are components of the system responsible for streaming and that it is an interaction between them which produces streaming. However, in *Nitella* the two components are separated by the width of the plastid layer, so that it is not clear how they could interact.

Secondly, the substructural microfilaments (50 to 70 Å wide), which may occur free in the ground substance (as in melanocytes) or as aggregates in fibers, might be in reality subunits of microtubules, in which case streaming might occur in relationship with these microfilaments whether they are part of a microtubule or not. In this connection, it is certainly noteworthy that both cortical microtubules[10] and axonemal fibers of sperm tails[16] have been shown to possess a subunit whose size is probably not significantly different from that recorded in these filaments. Thus, whether the microfilaments are organized in the ground substance as a microtubule or not might have little relevance *from the point of view of streaming;* it would, nevertheless, be quite important for functions associated with the establishment and maintenance of cellular asymmetry. On this hypothesis the microtubule would be envisaged primarily as a structure involved in controlling cell shape, and perhaps only secondarily involved in cytoplasmic movement by virtue of its component proteins. The advantage of having these functions combined in certain specific cases (e.g., in the axopods of *Actinospherium*[14] or the tentacles of *Tokophyra*[12]) is evident. This hypothesis also suggests that in suitable systems it might be possible to destroy the streaming function without destroying the structural function, and vice versa.

The location of the fibers in the center of the cytoplasm in vacuolated parenchyma cells agrees better with the location of the streaming cytoplasm than does the ex-

tremely peripheral location of the microtubules. On the other hand, the orientations of the fibers and microtubules raise interesting questions as to the way in which streaming might be interrelated with them. In parenchyma cells, the microtubules are generally oriented more or less parallel to the microfibrils in the last deposited layer of the cell wall,[17] i.e., approximately transverse to the long axis of the cell. The fibers, on the other hand, are largely parallel to the long axis. The direction of particle movement in cytoplasmic streaming is also, in elongated cells like the *Avena* coleoptile epidermis, almost wholly parallel to the long axis.[18] In cells that are more nearly isodiametric, streaming is often vigorous along the transvacuolar strands that connect the nucleus to the peripheral cytoplasm. It is significant in this connection that, as shown in Figure 7, these transvacuolar strands may contain well-developed longitudinal fibers. Thus, in these plant cells the fibers are parallel to the direction of streaming. In the slime mold the relations are not so clear; in the streaming cytoplasmic strands of *Physarum*, Wohlfarth-Bottermann's observations[8] seem to show that the fibers form largely perpendicular to the direction of streaming, and apparently disappear and reform with each reversal. The glycerinated plasmodium, when treated with ATP, contracts in the radial direction[19] which, by analogy with glycerinated muscle fibers, would indicate that the fibers are radial, i.e., parallel to the direction of streaming.

One other fact that supports a relationship between the fibers and cytoplasmic movement is the evident close association of mitochondria with the fiber surface (Figs. 1 and 7). This again suggests a contrast between the higher plant and the slime mold, for in the former, cytoplasmic streaming is known to depend on oxidations,[18] while in *Physarum* the motive force comes from glycolysis.[20]

Summary.—The epidermis and parenchyma cells of the oat coleoptile contain very long, fine fibers, 0.1 to 0.2 μ wide, which, when fixed in glutaraldehyde, are seen to be constructed of microfilaments 50 to 70 Å wide. Their orientation is parallel to the long axis of the cell, and longitudinal also in transvacuolar strands of cytoplasm. Evidence that the fibers are associated with cytoplasmic streaming is marshaled and discussed.

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