A SPINY VESICLE IN SLIME-PRODUCING CELLS OF THE BEAN ROOT

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Vesicles bearing a specialized layer associated with the outer surface of the bounding membrane have become widely recognized fine structural components since the first reports of their presence in animal cells (8, 13, 15). Presumably this morphological specialization of the vesicle, which usually takes the form of a hairy coating or an alveolate reticulum, is related to functional specialization, including selective absorption, transport, or fusion. In some instances it has been shown that these "coated" vesicles in animal cells are concerned in the selective accumulation and transport of protein (3, 7, 14).

Perhaps the first clear demonstration of coated vesicles in plant cells was that by Berkaloff (1) in the brown alga, *Himanthalia*. Manton (10) has illustrated and discussed fringed vesicles occurring in the green alga, *Stigeoclonium*, as have Leedale et al. (9) in *Euglena*. In the author's laboratory, vesicles with an alveolate surface and a diameter of about 85 mμ, including coat, have been observed in a variety of meristematic and differentiating cells in flowering plants and have been studied particularly in root hairs (2).

The present paper describes the occurrence of vesicles of a new kind in plant cells. These vesicles are unusual in several respects: they possess a specialized surface layer of a type not previously reported for vesicles in plant or animal cells, they occur in aggregates in the cytoplasm, and they are restricted to one stage in the differentiation of particular kinds of cells closely related ontogenetically.

MATERIALS AND METHODS

Seeds of bean (*Phaseolus vulgaris* L. var. Dwarf Horticulture) were obtained from Olds Seed Co., Madison, Wis. Following germination the plants were grown under greenhouse conditions for 3 wk in a modified Hoagland solution. Iron as ferric tartrate was added as needed to prevent chlorosis. Tips 2–3 mm long were cut from both main and lateral roots and fixed at room temperature for 1.5 hr in 3% glutaraldehyde containing 0.025 M phosphate buffer at pH 6.8. The root tips were then washed for 1 hr in four changes of 0.025 M phosphate buffer and postfixed in 2% osmium tetroxide in buffer of the same molarity for 2 hr. They were then dehydrated in an acetone series, and embedded in Araldite-Epon (11). Sections were cut on a Servall MT-1 Ultramicrotome with a diamond knife and mounted on 300 × 75 grids. The sections were then stained with aqueous 2% uranyl acetate followed by lead citrate (12), and viewed in a Hitachi HU-11A microscope at 50 kv with a 30 μ objective aperture.

OBSERVATIONS AND COMMENTS

Vesicles bearing numerous radiating projections have been observed during an investigation of cellular differentiation in root tips of bean. The vesicles appear to be confined entirely to the differentiating procambial cells bordering on protophloem sieve elements in the phloem strands (Figs. 1 and 2). Typically they occur in groups or clusters in the cytoplasm. Varying in both shape and size, they are irregularly spherical to ovoid and range in diameter from about 40 to 150 mμ, excluding projections (Figs. 1–3). Occasionally forms are seen that appear to be dividing or fusing (Fig. 2).

The projections from the bounding membrane, which are about 150 A apart center-to-center at their points of origin, appear to be distributed over the entire vesicle surface. They are cylindrical, with a length of about 325 A and a thickness of about 80 A. In both longitudinal view (Fig. 3) and transverse section (Fig. 4), they exhibit an electron-opaque cortex and transparent core; thus they appear to be tubular in nature. For convenience and brevity they will be referred to hereafter as "spines," and the vesicles bearing them as "spiny" vesicles, although it is recognized that these terms are not strictly accurate.

The spiny vesicles are further distinguished by their tendency to occur in clusters ranging from a

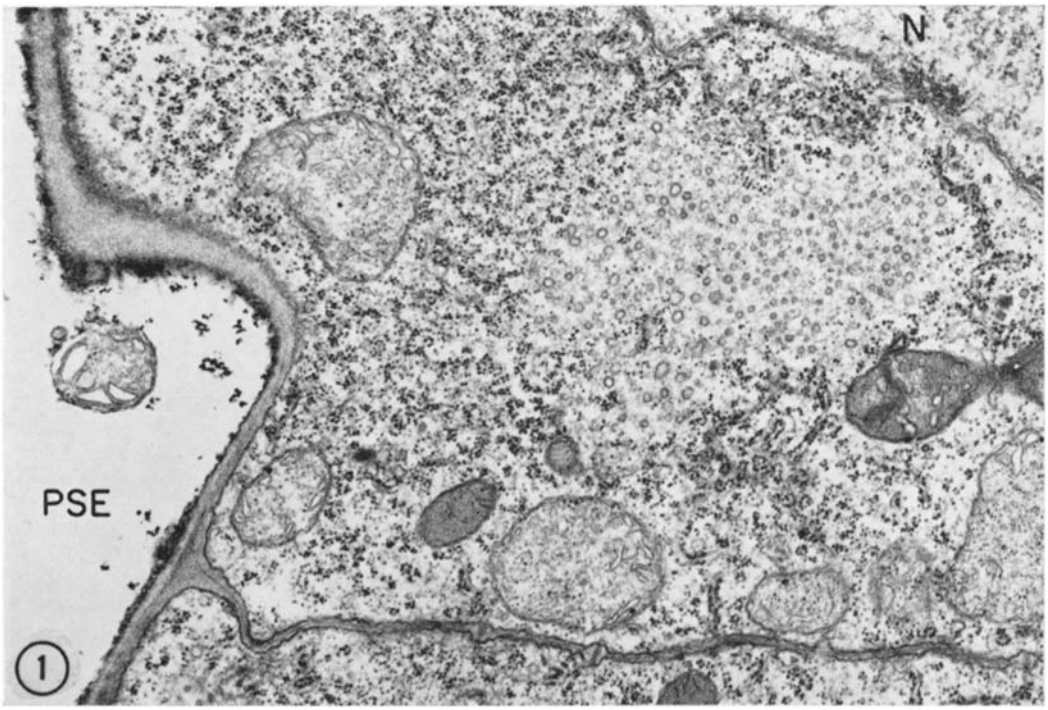

FIGURE 1 Longitudinal section of a bean root tip showing part of a cell adjacent to a protophloem sieve element (*PSE*). A large cluster of spiny vesicles lies near the nucleus (*N*). Mitochondria and plastids are also present. × 26,000.

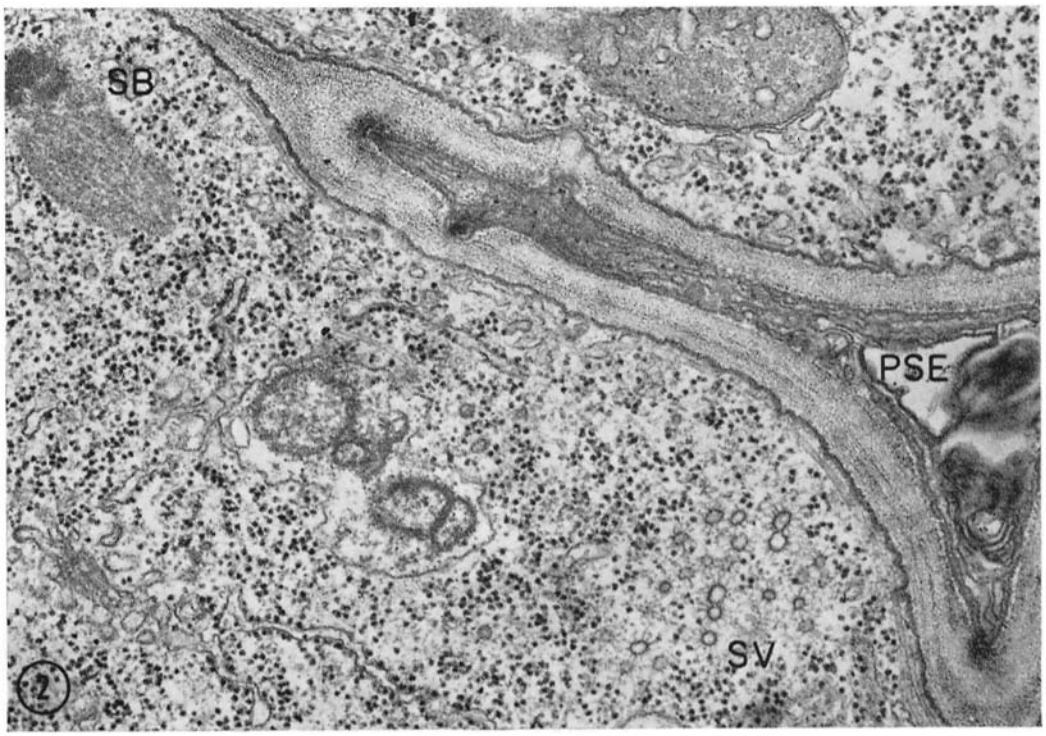

FIGURE 2 Transverse section of a bean root tip showing a portion of the cytoplasm in a cell adjacent to a protophloem element (*PSE*). A small cluster of spiny vesicles (SV) and a small slime body (*SB*) containing both tubular and fibrous components are present as well as fibrous material among the ribosomes. Microtubules conform to the curves of the thick nacreous wall of the sieve element. × 50,000.

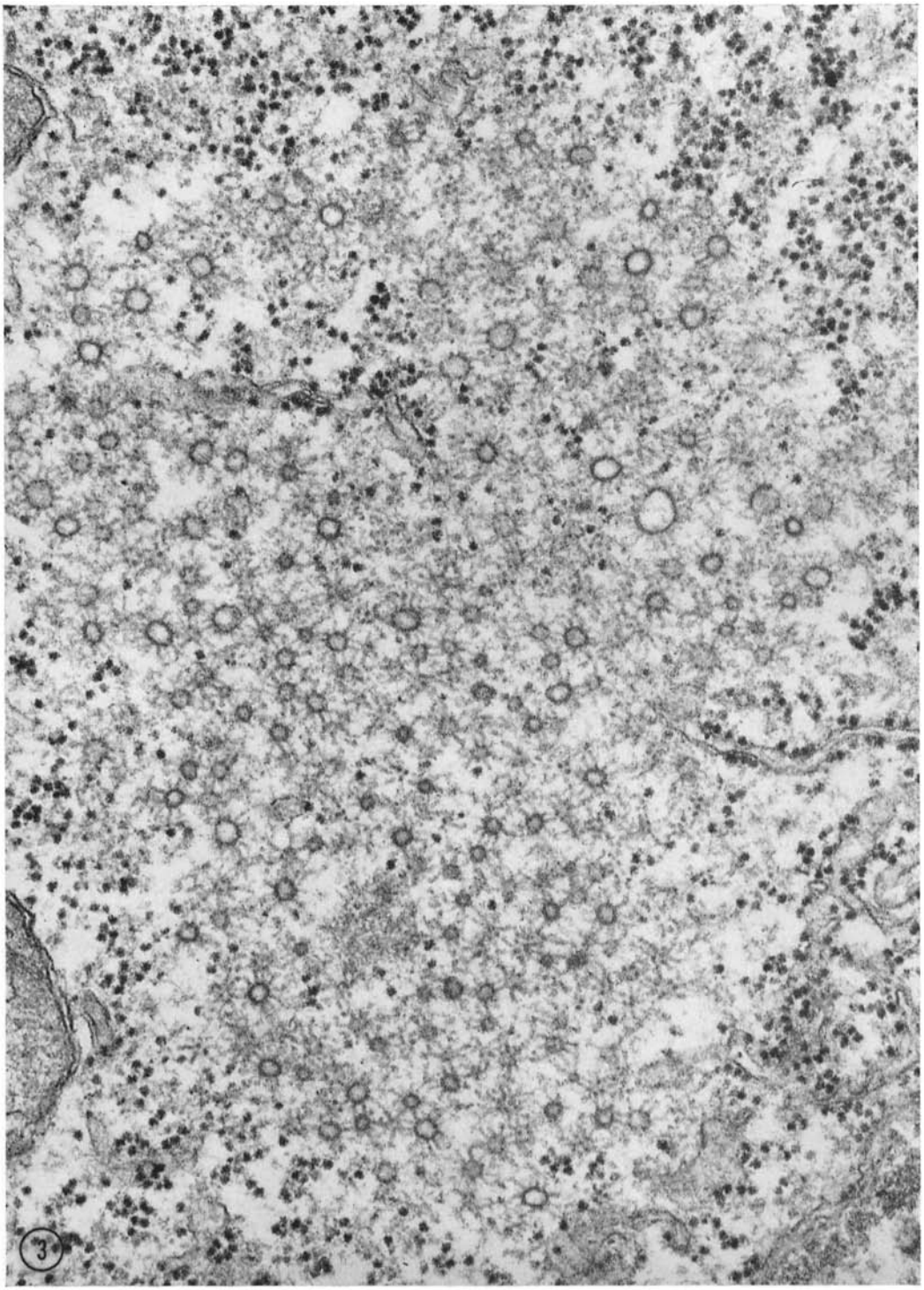

FIGURE 3 Section of a large cluster of spiny vesicles. Many vesicles are represented in the section only by spines. The ER profiles seen at upper left and lower right center may be continuous, their connection having been displaced from the plane of section by the mass of vesicles. × 73,000.

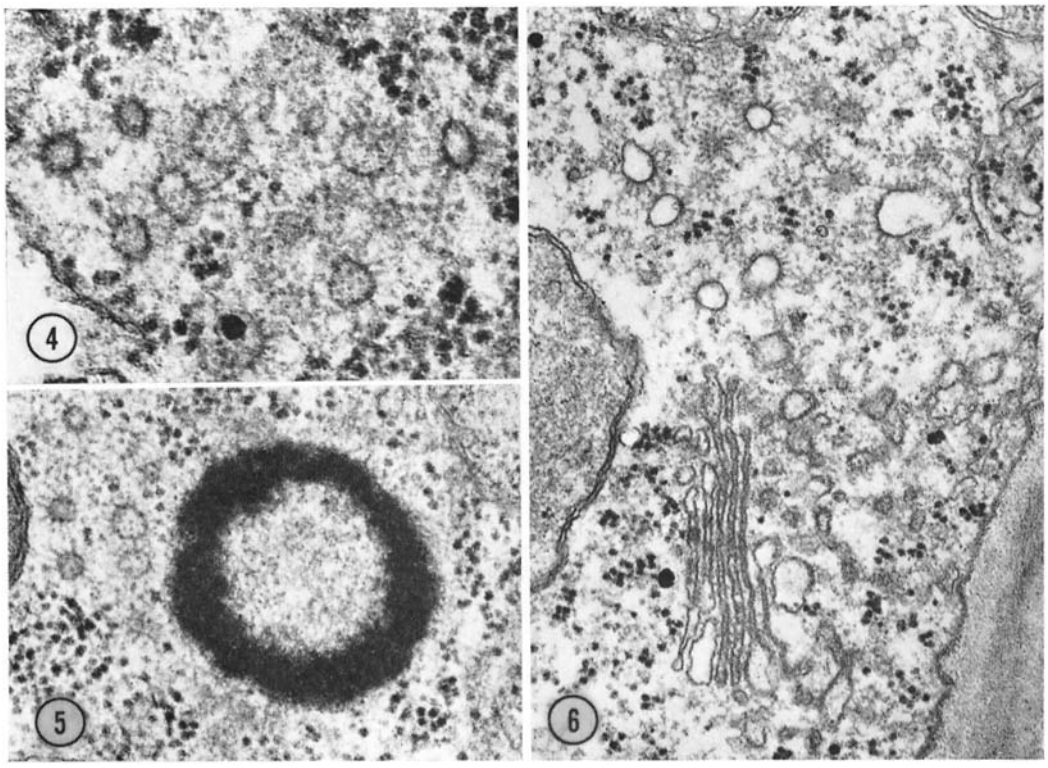

FIGURE 4 A cluster of spiny vesicles. The transversely sectioned spines appear tubular. × 100,000.

FIGURE 5 Early stage in the formation of a fibrous slime body. Several spiny vesicles lie in or near the finely fibrous material peripheral to the slime body. × 60,000.

FIGURE 6 Presumed origin of spiny vesicles from a dictyosome. Vesicles nearest the dictyosome lack spines and those somewhat farther away do not yet have spines differentiated over the entire surface. The spines of several vesicles have been sectioned transversely, and appear tubular. × 53,000.

few individuals to several hundred (Figs. 1–3). For example, parts of more than 300 vesicles can be detected in the section of the aggregate shown in Fig. 3. It seems probable that the vesicles lie embedded in a viscous matrix and remain associated for this reason. Although the space around the vesicles in a cluster appears structureless in the micrographs, it does possess a detectable degree of electron density (Fig. 3). Presence of a matrix is also suggested by the distribution of the vesicles within the clusters since there is considerable space not occupied by the spines, yet from which other cytoplasmic components, including ribosomes, are almost totally excluded (Figs. 1 and 3). Invasion of the outer parts of the larger clusters by numerous, dilated, mostly smooth-surfaced elements of ER has been observed repeatedly. Whether these elements are concerned in production of the vesicles or their spines is not clear.

It appears probable that the vesicles arise from dictyosomes. Although vesicles with well-defined spines have not been observed arising from the margins of dictyosome cisternae, clusters of the spiny vesicles have been seen frequently close to dictyosomes, and vesicles bearing a fuzzy, indistinct coat have been noted between the two, as in Figs. 6 and 7. This suggests that assembly of the spines may occur as a further stage of differentiation of material associated with the outer surfaces of the membranes after the vesicles separate from the dictyosomes.

Presumably a vesicle cluster forms during a period of intense dictyosome activity. Production of a viscous matrix either by a dictyosome or by

the vesicles themselves may cause them to accumulate and remain together. Although most of the spiny vesicles occur in aggregates, single vesicles as well as groups containing only a few members are also observed. These may represent products of a period of low dictyosome activity or occasional strays that escape during accumulation of a larger group.

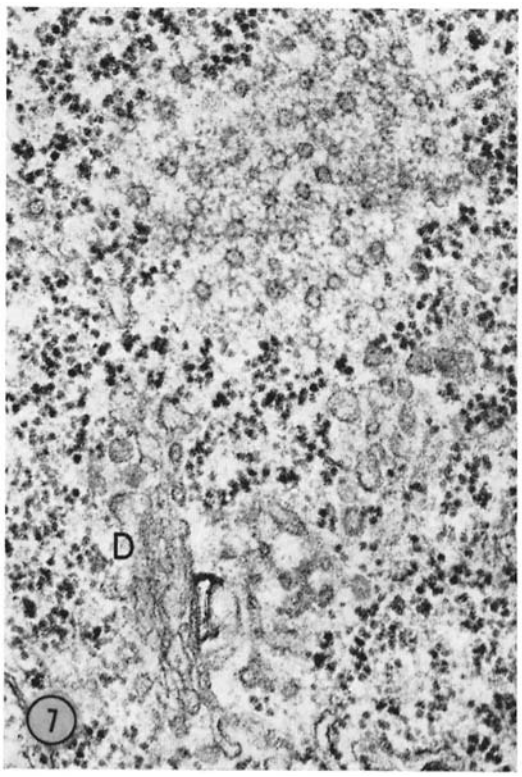

FIGURE 7 Cluster of vesicles believed to be differentiating spines from the associated material on their surfaces. Vesicles without spines in lower right center may be a group moving to the main cluster from the dictyosome (*D*). × 60,000.

The spiny vesicles are encountered within particular cells of the procambium that gives rise to a phloem strand. Within this procambium, the protophloem sieve elements with their thick, nacreous walls have a highly characteristic and readily identifiable structure (Fig. 2). The first protophloem element of a strand differentiates in a position midway between two future protoxylem poles and immediately beneath the pericycle. Subsequently, two more protophloem elements differentiate, one on either side of the first, and one cell removed from it. The spiny vesicles have not been detected in the differentiating protophloem sieve elements themselves; rather, they occur in the differentiating procambial cells of the phloem and pericycle bordering on the protophloem elements. In a detailed study of the fine

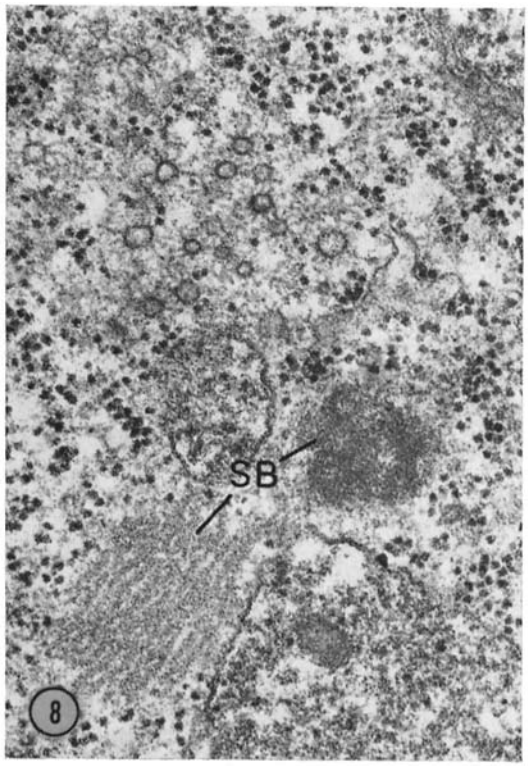

FIGURE 8 Small fibrous and tubular slime bodies (*SB*) in an early stage of development. Fibrous material and spiny vesicles are in the vicinity. × 60,000.

structure of the bean root tip, no exception to this generalization has yet been found.

The same procambial cells that form the spiny vesicles also produce another distinctive component, a proteinaceous "slime." This substance is particularly characteristic of the sieve elements of dicotyledons and is of great interest to students of metabolite transport in the phloem (4, 6). During slime production the cytoplasm is characterized by an abundance of free ribosomes and the accumulation among them of a finely fibrillar

matrix imparting a cloudy appearance to the cell contents (Figs. 2 and 8).

Two kinds of slime body appear in the cytoplasm of these cells. One consists of a dense mass of fine, electron-opaque threads or fibers that eventually becomes an extremely dense, spherical body ranging up to several microns in diameter. Relatively small, early stages of this kind are present in Figs. 2 and 5. The second kind consists of a spindle-shaped bundle of tubular strands, each strand approximately 130–140 A in diameter. Examples of small forms of the tubular kind, not yet well developed, are seen in Figs. 2 and 8.

The spiny vesicles are closely related to the slime bodies both in time and in place of occurrence: they appear in the same cells before and during slime body formation and disappear as the bodies enlarge. They are seen occasionally in the less dense peripheral material of aggregating bodies of the fibrous kind, where they usually appear indistinct, as though undergoing dissolution (Fig. 5). They are also sometimes present in small numbers at the margins of slime bodies of the tubular kind. These observations suggest that they may contribute to formation of the slime bodies. There is, on the other hand, a strong indication, based on numerous micrographs, that the slime bodies arise at least in part by aggregation of the aforementioned finely fibrous material present in the cytoplasm at this stage. Fig. 8 is suggestive, but also ambiguous, since it shows not only large amounts of this material but also a group of spiny vesicles near both kinds of developing slime body. It is not excluded that the fibrous material, or a portion of it, arises by breakdown of the spiny vesicles.

At present, then, the evidence for the participation of spiny vesicles in the formation of slime bodies is inconclusive despite their restriction to the cells in which slime is being formed. Regarding other possible fates of the spiny vesicles, there is no indication that they migrate to the cell surface and fuse with the plasmalemma, as appears to be the case with coated vesicles of the alveolate kind (2), which are also present in these cells.

It has been found in this investigation that differentiating pericycle cells share some unusual, fine structural features with nearby procambial cells destined to become phloem. These include the presence not only of spiny vesicles but also of slime bodies, which do not appear to have been reported previously in the pericycle. These results are perhaps not surprising since the cells of the pericycle are also derived from the procambium and thus are closely related ontogenetically to the cells in the phloem strand (5).

Spiny vesicles similar to those described in this paper have also been seen in this laboratory in differentiating cells of the phloem strands of *Dianthus* roots and *Coleus* petioles. These observations encourage the view that these distinctive vesicles will prove to be of considerable morphogenetic interest.

This work was supported in part by Grants GB-628 and GB-6161 from the National Science Foundation.

Received for publication 18 August 1967.

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