Orion Nebula. Presumably the shock front has so small a radius there that the inner section of the H II region cannot be resolved. With a limit of resolution of 3 minutes of arc and an estimated distance of 500 pc between us and Orion, this means that the radius of the shock must be less than 0.5 pc, so that

$$R_s = a_c t/\eta_s < 0.5 \text{ pc} = 1.5 \times 10^{18} \text{ cm}.$$

Since $\eta_s = {}^1/_4$ and $a_c \doteqdot 1.4 \times 10^6$ cm/s, it follows that the age t of the ionized region must be less than 3×10^{11} s, or 10,000 years. In the Rosette Nebula $R_s \doteqdot 2.45$ pc; the corresponding age t is about 50,000 years.

The age of the Trapezium Cluster in the Orion Nebula has, for comparison, been variously estimated to be 10,000 years and up to 300,000 years by Parenago[3] and by Strand.[4] The cluster NGC 2244 immersed in the Rosette Nebula has not been studied in sufficient detail to provide us with a reliable estimate of its age. The present investigation seems to indicate that dense H II regions tend to be extremely young objects.

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† Operated by Associated Universities, Inc., under contract with the National Science Foundation.

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AN EVALUATION OF STUDIES ON ULTRASTRUCTURE OF SIEVE PLATES

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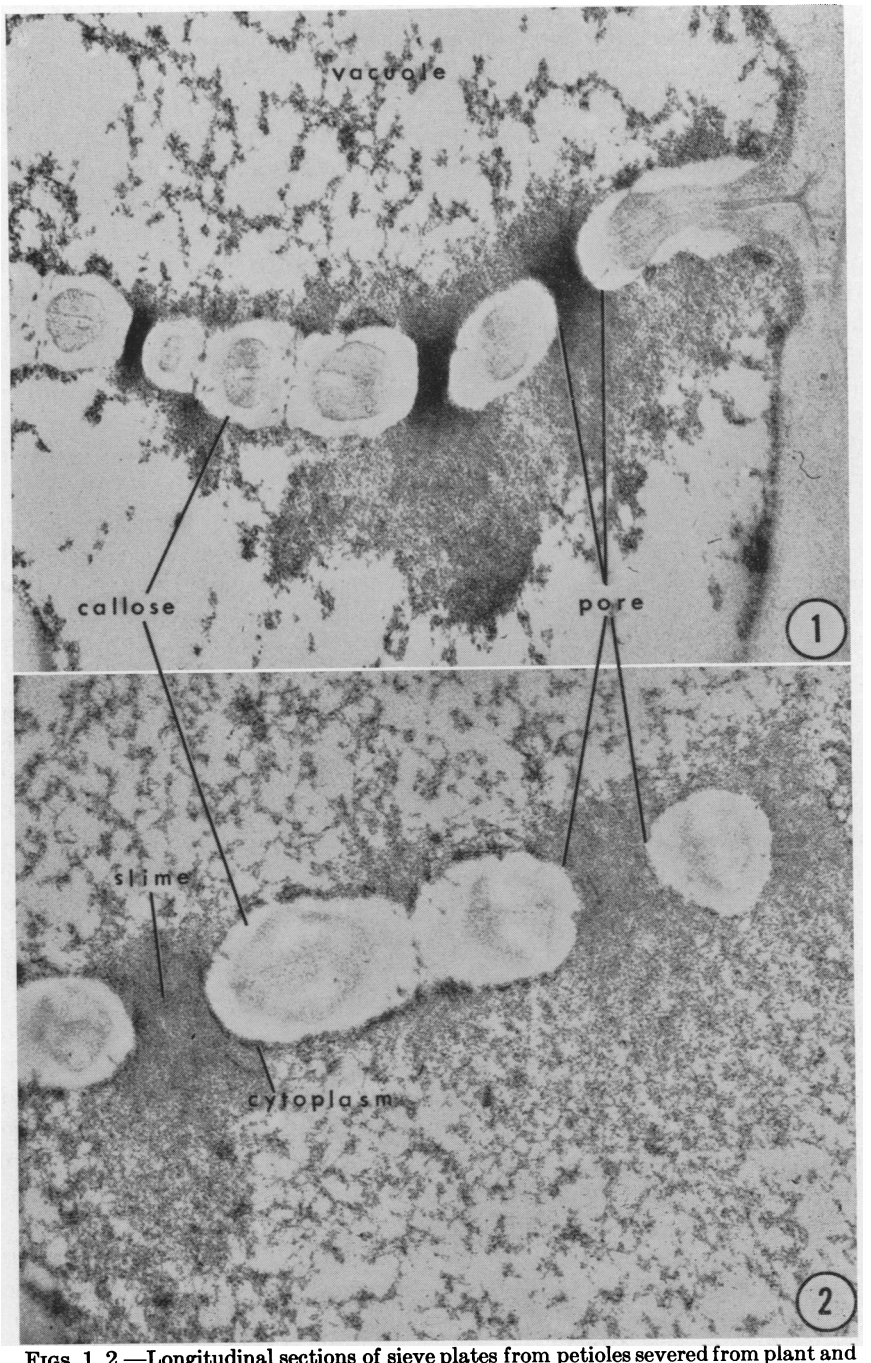
Botanists interested in the translocation of organic solutes in plants are constantly seeking support for their concepts of this phenomenon in the structure of the presumed principal conduit in the phloem tissue, the sieve element. Electron microscopy offers a means of extending this search.

Among the various features of the sieve element, the strands connecting the superimposed sieve elements with one another through the sieve plates and the limiting layer between the cytoplasm and the vacuole (that is, the tonoplast) are receiving especial attention because the nature of these structures might be casually related to the ability of phloem tissue to conduct the solutes at the observed high velocities. Absence of the tonoplast and some peculiar structure of the connecting strands—for example, a structure permitting an intervacuolar connection—would enable one to think of the translocation in terms of mass flow; lack of such specializations would suggest support for the concept of molecular movement.



FIGS. 1, 2.—Longitudinal sections of sieve plates from petioles severed from plant and killed in potassium permanganate. Fig. 1 ×18,500; Fig. 2 ×24,700.

Electron microscopy is now being used to determine the fine structure of the cytoplasmic membranes of sieve elements and of the connecting strands in the sieve plates.

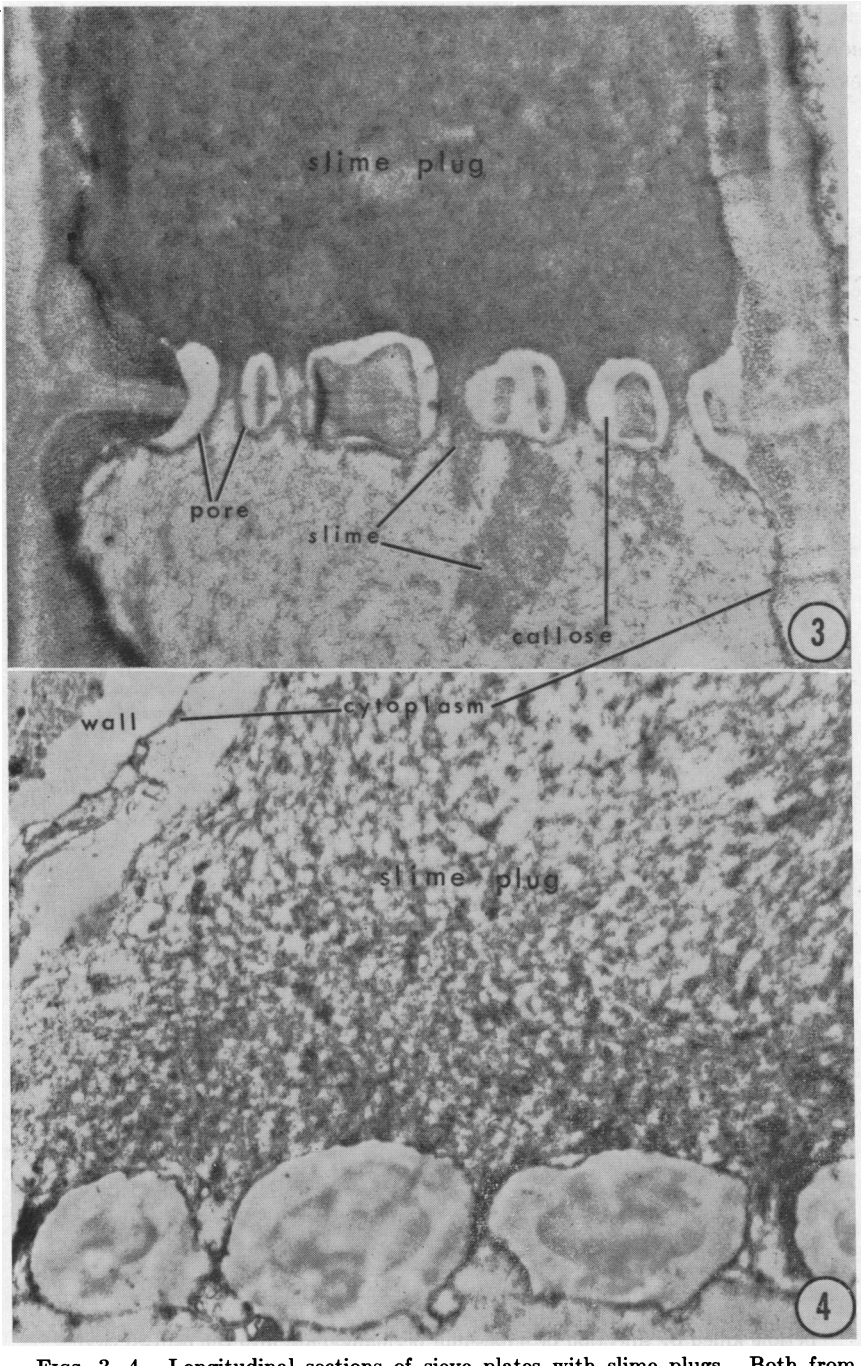
The present paper considers the published descriptions of the ultrastructure of the sieve plates with reference to our own studies on the sieve elements of *Cucurbita*. A paper to follow deals with the tonoplast.

Material and Methods.—*Cucurbita maxima* Duchesne (winter squash) grown in a greenhouse provided the material for study. Several fixation and embedding methods were used. The sections that served for the electron micrographs in this paper were obtained from material treated as follows: Figures 1 and 2 from 2-inch-long petiole cut and evacuated under tap water and fixed for 5 minutes in 4 per cent $KMnO_4$ solution in water; Figure 3 from a 4-inch-long petiole cut and evacuated under tap water and fixed for 18 hours in a solution of $^1/_2$ gram osmium tetroxide in 25 cc of 4 per cent $K_2Cr_2O_7$ at pH 7.0; Figure 4 from an internode at base of plant sectioned in a 0.25 *M* sucrose solution and fixed for 5 minutes in 2.5 per cent $KMnO_4$ dissolved in a 0.3 *M* NaCl solution; Figures 5, 6, 9, and 10 from 4-inch-long petiole fixed for 10 minutes *in situ* on the plant by injection of a 4 per cent $KMnO_4$ solution in water into its central cavity. All material was treated similarly after fixation: dissected in a 30 per cent ethyl alcohol, dehydrated in a graded series of alcohols at 1–2-hour intervals, and embedded in methacrylate which was prepolymerized under an incandescent lamp after addition of 1 per cent α,α-azodiisobutyronitrile. All fixations were carried out at room temperature.

The interpretations of the constituents of the connecting strands in the illustrations are tentative.

Critique of Literature and New Observations.—The studies on the ultrastructure of sieve plates published thus far agree that the connecting strands in the highly differentiated sieve areas of the sieve plates are continuous from cell to cell but that they consist of electron dense material giving no certain indication of vacuolar continuity (comparable view in Fig. 1). Papers from Leeds, England,[1, 2] show sieve plates of *Cucurbita*, *Sorbus*, and *Vitis* with dense connecting strands more or less continuous with the deeply stained material located on one or both surfaces of the sieve plate. The authors concerned regard the dense staining of the strands as particularly significant; because of this feature the strands may not be ordinary cytoplasmic bridges and, thus, strictly speaking, not plasmodesmata. The stainability of the strands with osmic acid is taken as an indication of presence of unsaturated fats. The Leeds authors correlate this indication of fats with the reports in the literature that the ectoplast (plasma membrane) may contain lipids and that plasmodesmata are extensions of the ectoplast; they suggest that the connecting strands are interpretable as products of fusion of numbers of such extensions of the ectoplast (that is, of plasmodesmata) in which the lipoidal substances are responsible for the dense staining.

Schumacher and Kollmann[3] illustrate dense connecting strands in *Passiflora* and stress their continuity with the parietal cytoplasm (actually they refer to the densely stained material covering the sieve plate; cf. Figs. 1, 2, in this paper). In Kollmann's[4] further study of *Passiflora*, some especially thin longitudinal sections made possible the resolution of the connecting strands into fibrils 100–150 Å in thickness, each apparently constituting a double membrane or a tubule. These fibrillar elements were seemingly connected with similar elements stretching from the sieve plate on into the lumen of the cell. Ziegler[5] observed fibrillar elements in the cell lumen and on the sieve plate in *Heracleum* but could not identify them with



FIGS. 3, 4.—Longitudinal sections of sieve plates with slime plugs. Both from material severed from plant before fixation.

Fig. 3, osmium tetroxide and potassium dichromate; Fig. 4, potassium permanganate with the addition of NaCl

Fig. 3 × 21,000; Fig. 4 × 16,675.

certainty in the connecting strands. Hohl[6] depicted similar material in a sieve element of *Datura* and called it lamellar cytoplasm.

Several of the authors mentioned above[1-4] developed the thought that the dense

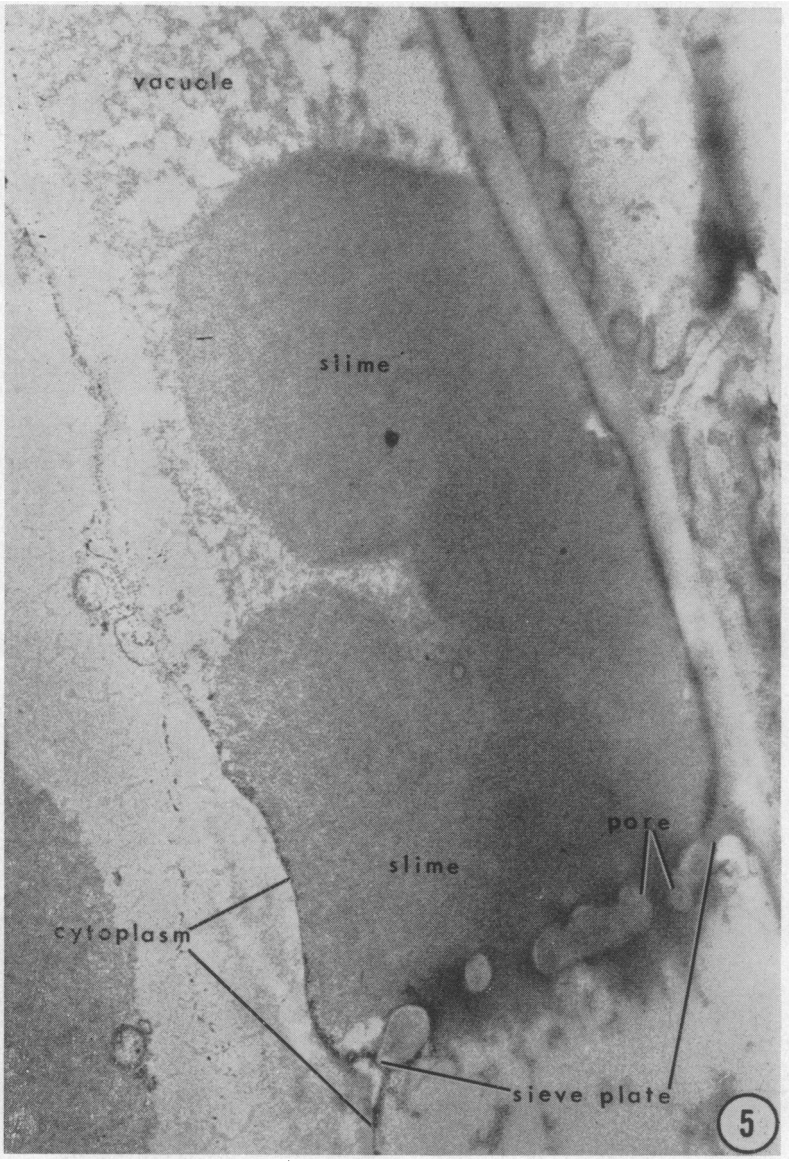


FIG. 5.—Longitudinal section of part of sieve element showing sieve plate occluded by a slime plug in which component slime bodies are discernible. From petiole killed by injection of potassium permanganate into the central cavity. × 9,000.

structure of the connecting strands precludes the possibility of mass flow through the sieve plates. Kollmann[4] added that the occurrence of fibrillar elements possibly indicates a special differentiation of cytoplasmic surfaces that might play a

role in the transport of molecules in the sense of absorption and movement along interfaces. Hohl[6] also suggested a molecular movement along the interfaces of the lamellar material seen in *Datura*.

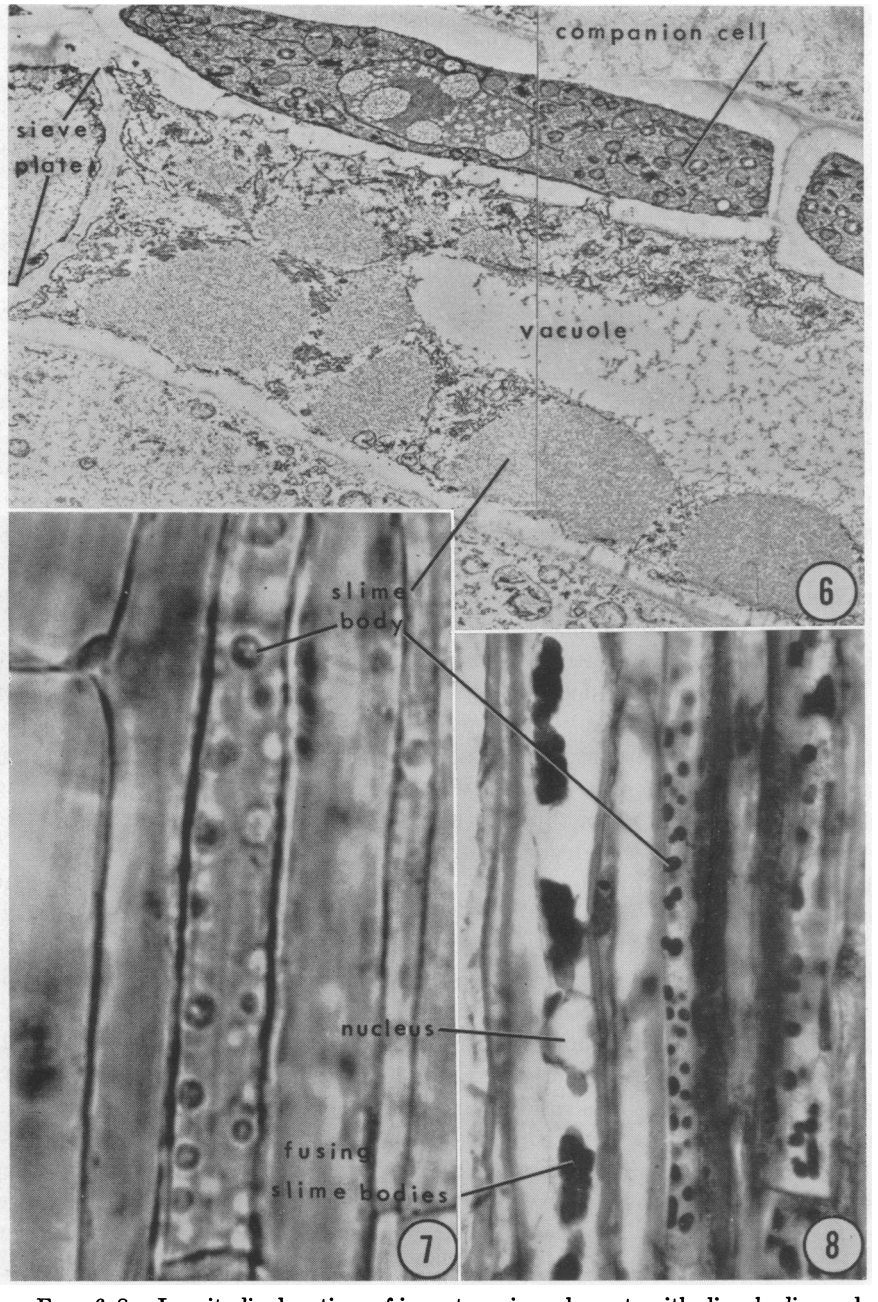
Ziegler,[5] on the other hand, compared the fibrillar elements with the endoplasmic reticulum and suggested that if the lamellae of this reticulm are continuous through the pores of the sieve plate, the connecting strands would have a submicroscopic pore system providing the microcapillary system required for a mass flow in the sense of Crafts.[7] In addition, Ziegler suggested that finer capillaries between adjacent lamellae are perhaps considerably reduced in the tightly packed connecting strands so that the free movement of long molecules like those of sucrose is impeded in these capillaries and a molecular acceleration by interfacial flow may occur.

The suggestion that the connecting strands might consist of complexes of membranes connected with similar membranes in the lumen of the cell is so far the most arresting concept that has been presented in articles on the ultrastructure of sieve elements. It parallels the idea that the endoplasmic reticulum is continuous through the plasmodesmata.[8] One must bear in mind, however, that to obtain images with the electron microscope the material must be killed and dehydrated, that is, considerably denatured. The pertinent question is, therefore, to what extent does the structure seen with the electron microscope resemble that present in the living cell within the intact plant.

The sensitivity of the mature sieve elements even to minor disturbances that may not affect ordinary nucleate cells in the same tissue has been sufficiently emphasized in the literature. The studies reviewed above, except that of Hohl,[6] were based on material prepared so as to minimize effects of such sensitivity: before fixation the material was either treated with a sugar solution to reduce the damaging effect of the sudden release of turgor in the cut tissue or, in one study,[5] the phloem strand was killed by freeze-drying while it was still attached to the plant. The resulting illustrations show no conspicuous slime plugs, that is, unilateral accumulations of slime on the sieve plates (cf. Figs. 3–5), which are considered to be a reliable sign of injury to the sieve element.

The idea that the slime-plug formation is a response to injury is generally accepted, but the absence of a plug cannot be taken necessarily as evidence that the cell has not been denatured to any degree. Therefore, the tendency of the cell to respond to injury by a displacement of slime must be taken into consideration when the nature of the connecting strands is discussed. It bears repetition that the slime, though still not fully investigated chemically, is a real component of the sieve-element protoplast in the dicotyledons. It originates in young cells, becomes aggregated into one or more discrete bodies—these are readily seen in living, still nucleate cells (Fig. 7), in material processed through paraffin (Fig. 8), and in electron micrographs (Fig. 6)—and then later disaggregates. Thus in mature, living, not obviously injured sieve elements the slime is usually not discernible (except during dormancy as in *Vitis*).[10] In fixed mature sieve elements it is best known in its reaggregated form, the slime plug (Fig. 3), and investigators tend to disregard the occurrence of slime when no such plug is in evidence.[4]

If one seeks to determine the nature of the connecting strands, he must first find answers to two basic questions regarding slime: first, where is the disaggre-



FIGS. 6–8.—Longitudinal sections of immature sieve elements with slime bodies and of adjacent phloem cells as seen in an electron micrograph (Fig. 6), in living state (Fig. 7), and in material sectioned in paraffin and stained with Heidenhain's hematoxylin (Fig. 8). The fusion of slime bodies (Fig. 8) precedes their dispersal in the vacuole. The nucleus (Fig. 8) is partly disintegrated. Fig. 6 × 4,240; Fig. 7 × 2,200; Fig. 8 × 650.

gated slime located in an uninjured cell; second, in fixed sections, is the dense material, visible on the sieve plate and within its pores, free of slime when no conspicuous slime plug is present.

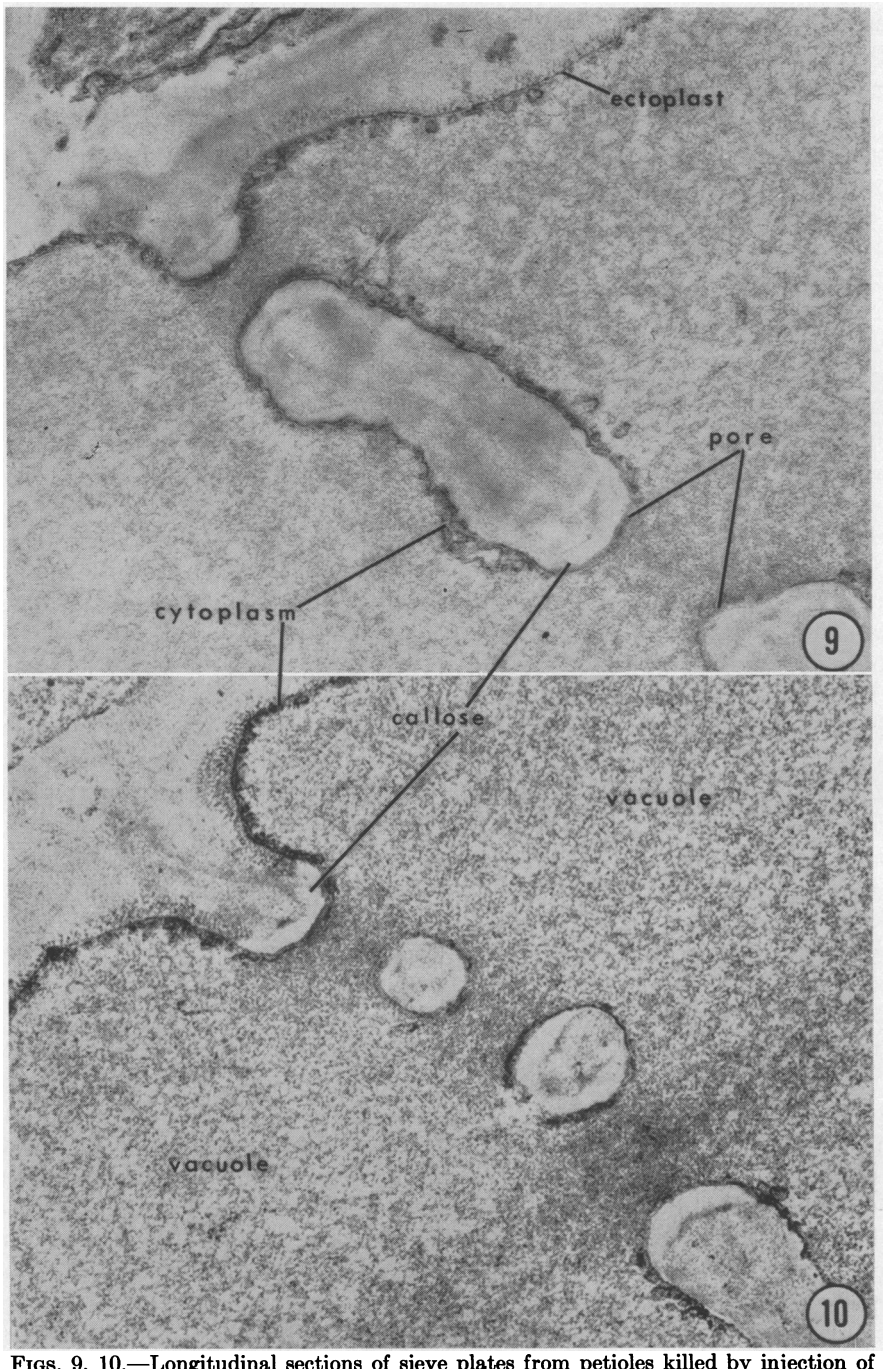
The slime bodies of young cells are definitely restricted to the parietal layer of cytoplasm (Fig. 6); the more or less disaggregated slime comes to occupy the central part of the lumen of the cell (transitional stage—bodies fusing—to the left in Fig. 8) while the cytoplasm is reduced to an exceedingly thin layer lining the wall (Figs. 9, 10). It is highly probable that the slime becomes a component of the vacuole in the mature sieve element. When a slime plug is formed, the disaggregated slime of the vacuole reaggregates on the sieve plates. In this form it may or may not be distinguishable from the parietal cytoplasm enclosing the slime plug (Figs. 3, 4). Although the slime plug usually results from reaggregation of the dispersed slime, it may be formed before the disaggregation of slime bodies is completed. Then the slime plug consists of a mass of partly fused slime bodies (Fig. 5). In certain Leguminosae the slime body does not disperse and forms the slime plug as a unit.

The second question has less visual evidence for an answer than has the first. As was mentioned, slime bodies and massively reaggregated slime may be distinguished from the cytoplasm (Figs. 4–6), and the slime plug may have extensions within the pores (Figs. 3–5). Would one still distinguish between the slime and cytoplasm if only small amounts of slime were to accumulate on the plate (Figs. 1, 2)? If the slime were a component of the vacuole and the mature sieve element had a tonoplast, then perhaps one could delimit the slime from the cytoplasm. The presence of a normal tonoplast in mature sieve elements, however, has not been demonstrated unequivocally by light microscopy, and published electron micrographs[1–6, 9] suggest a gradual fading out of the material on the sieve plate into the central vacuolar space (comparable to Figs. 1, 2 in this paper).

The pretreatment with sugar solutions, which reduces the frequency and intensity of slime-plug formation, proved most helpful for demonstrating the plasmolyzability of the sieve-element protoplasts in a variety of species.[10] This result does, indeed, indicate that the pretreatment reduces injury to the sieve element. Nevertheless it does not assure that the sieve plate is in no way affected by the manipulations. Rouschal,[11] for example, suggested that the plasmolyzability of the sieve elements can be demonstrated only if the pores are plugged by coagulated protein and each sieve element behaves as an independent cell rather than as a member of a series of elements (sieve tube) connected by permeable sieve plates. We do not yet know how precisely to determine the role of slime and its relation to the cytoplasm and how to explain its various manifestations.

The techniques for electron microscopy have not progressed to the stage that tissues of the higher plant can be examined for their ultrastructure in living state. The only possible course at present is to gather extensive information on a variety of materials treated in a variety of ways. The illustrations in this paper give examples of some of the striking variations in the ultrastructure of the sieve plates of *Cucurbita* that may be obtained with different treatments. In fact, even material subjected to one treatment may show profound differences in different cells.

Figure 3, obtained from material killed with a combination of osmium tetroxide and dichromate, shows a typical slime plug with extensions into the pores of the



FIGS. 9, 10.—Longitudinal sections of sieve plates from petioles killed by injection of potassium permanganate into the central cavity. Fig. 9 × 28,500; Fig. 10 × 24,700.

sieve plate. In the lower cell, the slime merges with the vacuolar material. A thin parietal layer that appears to be the cytoplasm is evident on the lower side of the sieve plate and along the wall to the right in the lower cell. Figures 1, 2, and 4,

from material killed in potassium permanganate (the piece to be killed was severed from the plant before the treatment), show variations in the amount and density of accumulations, probably of slime, on the sieve plate. In Figure 1, the material in the pores and on the sieve plate permits no distinction of a parietal layer of cytoplasm, but Figure 2 does suggest a distinction between parietal cytoplasm and the bulk of the accumulation. In Figure 4 the slime plug has a fibrillar appearance and is somewhat contracted, so that a space is left between it and the parietal layer of cytoplasm.

The clearest difference between the parietal cytoplasm and the material filling the pores in the sieve plates was found in the material killed by the injection method with $KMnO_4$ (Figs. 9, 10). The cytoplasm was resolvable into a thin, almost continuous layer next to the wall (here interpreted as ectoplast) and a discontinuous layer of bodies and fragments that appeared to be the remnants of the various organelles and membranes usually recognized in meristematic cells,[8] and also present in companion cells and immature sieve elements (Fig. 6). This discontinuous layer showed no boundary, that is, tonoplast, toward the vacuole. The parietal cytoplasm was continuous from cell to cell through the pore; in other words, the pores were lined with cytoplasm. The interior of the pores, however, was filled with material that was slightly denser than the material in the vacuoles but completely continuous with it. Some of the sieve plates from the injected petioles showed slime plugs in which the distinction between the cytoplasm and the vacuolar material was obscure at the sieve plate (e.g., Fig. 5).

In contrast to the sieve plates, the lateral sieve areas, in which the connecting strands are considerably thinner than in the sieve plates, showed apparently solid dense strands, hardly suggesting any vacuolar continuity.

If an attempt were made to relate the structure of the sieve plate to its function, one could select Figures 9 and 10 to suggest a complete vacuolar continuity between superposed sieve elements; a mass flow of solutes could be readily visualized through such sieve plates. A view like that in Figure 1, on the other hand, does not suggest a continuity of vacuoles; rather, it agrees with the published reports that the connecting strands are solid structures. We need to know which of the different views reflects the conditions in the intact plant.

The closest approximation to the fibrillar or lamellar structure of the material on the sieve plate, as depicted by Kollmann,[4] Ziegler,[5] and Hohl,[6] appears in Figure 4. This material is here interpreted as slime. Buvat's[9] Figure 8 also shows that the fibrillar material is part of the slime plug. In fact, Kollmann[4] reported that the slime reaggregations which he saw in poorly fixed sieve elements of *Passiflora* also consisted of fibrillar elements, but he used this observation in concluding that the slime reaggregations were artifacts of cytoplasmic origin. Hohl,[6] however, specifically stated that the lamellar material was not slime. Obviously, the recognition of the true nature of the fibrillar or lamellar material in the sieve elements must await more diversified studies, including the developmental aspects of the sieve-element protoplast.

Conclusion.—The connecting strands of the sieve plates of *Cucurbita* have a variable appearance under the electron microscope. Some views suggest intervacuolar connection between sieve elements through strands that appear to have an outer layer of cytoplasm and an inner core of vacuolar material; others show

solid connecting strands. These variations may be traced, in part, to methods of preparation of the material. The papers published thus far show only solid connecting strands or those resolvable into a system of fibrillar elements. Before the attempt is made to relate the ultrastructure of the sieve plate to the movement of solutes in the phloem one needs to know which view illustrates most accurately the condition in the intact plant. The composition of connecting strands in sieve plates cannot be fully determined until the relation between slime and cytoplasm in mature sieve elements is understood.

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*IN SITU DETECTION AND ESTIMATION OF CHLOROPHYLL AND OTHER PIGMENTS IN THE OCEAN**

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The scattering centers in ocean water and in other natural scattering-absorbing media give rise to space light in the medium. This space light contains quantitative information about the absorption spectra of the components of the medium. By appropriate spectral measurements this information can be obtained in a form which may be useful for estimating the standing crop of phytoplankton in the ocean as a function of location or time, for *in situ* identification of plankton communities, for comparing and classifying waters with respect to clarity and productivity, for measuring the absorption spectra of living marine organisms in their natural environment, and for studying various other aspects of optically extensive scattering-absorbing media.

Theory.—In a source-free homogeneous scattering-absorbing medium, the change in field radiance, N_r, as a function of position, r, at some point along any path of sight, is given by the equation of transfer for radiance.[1]

$$\frac{dN_r}{dr} = -\alpha N_r + N_* \qquad (1)$$