P PROTEIN IN THE PHLOEM OF *CUCURBITA*

II. The P Protein of Mature Sieve Elements

JAMES CRONSHAW and KATHERINE ESAU

From the Department of Biological Sciences, University of California, Santa Barbara, California 93106

ABSTRACT

During maturation of sieve elements in *Cucurbita maxima* Duchesne, the P-protein bodies (slime bodies) usually disperse in the tonoplast-free cell. In some sieve elements the P-protein bodies fail to disperse. The occurrence of dispersal or nondispersal of P-protein bodies can be related to the position of the sieve elements in the stem or petiole. In the sieve elements within the vascular bundle the bodies normally disperse; in the extrafascicular sieve elements the bodies often fail to disperse. Extrafascicular sieve elements showing partial dispersal also occur. The appearance of the sieve plate in fixed material is related to the degree of dispersal or nondispersal of the P-protein bodies. In sieve elements in which complete dispersal occurs the sieve plate usually has a substantial deposit of callose, and the sieve-plate pores are filled with P protein. In sieve elements containing nondispersing P-protein bodies the sieve plate bears little or no callose, and its pores usually are essentially "open." The dispersed P-protein components may aggregate into loosely organized "strands," which sometimes extend vertically through the cell and continue through the sieve-plate pores; but they may be oriented otherwise in the cell, even transversely.

INTRODUCTION

The preceding paper (4) was concerned with the formation of P-protein bodies and the fine structure of the P protein (slime) in *Cucurbita maxima* Duchesne. *Cucurbita* sieve elements contain two types of P-protein bodies differing in the nature of the P-protein components. The development of these bodies during the differentiation of the sieve element was investigated and was compared with that of the P-protein bodies in *Nicotiana tabacum* (see reference 3).

The usual ontogeny of the P-protein bodies (see reference 5) may be briefly summarized. The bodies originate in the cytoplasm and increase in size, first apparently by addition of substance and later by a form of swelling or hydration. The swelling introduces the stage of dispersal of the P protein which is a concomitant of certain other significant developmental changes in the protoplast, the degeneration of the nucleus and the breakdown of the tonoplast. The P-protein bodies disperse, and their components apparently become evenly distributed in the cell, although in mature cells the P protein commonly accumulates at one or both sieve plates presumably in response to injury to the cell.

The present investigation has shown that the sequence just outlined does not invariably take place. In certain sieve elements the P-protein bodies fail to undergo the swelling and dispersal phenomena even though the nucleus and tonoplast break down as usual.

The terminology for the P-protein components has been introduced in previous papers (3, 4, 6), and we propose to continue using the terms.

MATERIALS AND METHODS

The material and its processing were the same as described in the previous paper (4). *Cucurbita maxima* Duchesne plants were grown from seed under greenhouse conditions. 1 cm pieces were cut from young petioles and internodes and were placed in glutaraldehyde-formaldehyde according to the method of Karnovsky (10). These samples were cut into smaller pieces after ½ hr, and fixation was continued for a total of 2 hr. Postfixation was carried out with 2% osmium tetroxide. The material was dehydrated through acetone solutions and embedded in Epon epoxy resin. Sections were cut with a diamond knife on a Porter-Blum MT 2 ultramicrotome, stained with uranyl acetate and lead, and viewed and photographed with a Siemens Elmiskop I.

Comparable material was fixed for the light microscope in a Craf III mixture (12) or 10% acrolein and embedded in Paraplast (Scientific Products, Inc., Detroit, Michigan). For developmental studies, the sections were stained with Heidenhain's hematoxylin.

OBSERVATIONS

In reexamining the behavior of the P protein during the maturation of the sieve element, we observed a deviation from the usual sequence of complete dispersal of this material. Observations with the light and the electron microscopes showed that in some sieve elements the P-protein bodies failed to disperse and remained identifiable as such in mature and senile cells. A survey of the maturation phenomena in different parts of the phloem system revealed a relation between the nondispersal of P protein and the position of the sieve elements in the system. The phloem system of *Cucurbita* is highly complex; it consists not only of components of the bicollateral vascular bundles but also of sieve tubes and companion cells which form a network outside the bundles. This extrafascicular phloem was first described by Fischer (9) and was reexamined by Crafts (2). The extrafascicular sieve tubes occur on the inside and on the outside of the cylinder of sclerenchyma and are connected with the sieve elements of the external and internal phloem of the bicollateral bundles. In Crafts' (2) illustrations, the extrafasicular sieve elements nearest the vascular bundles are seen to be in arcs on the periphery of the phloem. Crafts apparently assumed these cells to be elements of the phloem of the bundles and called them peripheral sieve tubes. Ontogenetic studies have demonstrated that they are members of the extrafascicular system (1).

Crafts (2) observed cytologic differences between the fascicular and the extrafascicular (including the "peripheral") sieve elements in early development. Both kinds of sieve elements contain slime bodies, although those of the extrafascicular system are somewhat smaller. In the fascicular sieve elements the slime bodies undergo the usual metamorphosis and eventually disintegrate into a "colloidal suspension in the vacuole" (2, p. 189). In the extrafascicular sieve elements the vacuolar contents become granular and later amorphous. Crafts observed no slime plugs in these sieve elements and found that callose deposition was less common than that in the fascicular elements. From this discription it is not clear what happens to the slime bodies and whether the granular and later amorphous material is derived from them. The apparently sole indication that nondispersal of P-protein bodies in *Cucurbita* was previously observed is found in Strasburger's report (13). In Fig. 25, plate IV, of his book, Strasburger depicted a small vein with no abaxial phloem but with a wide sieve tube on the adaxial side. In the pertinent text he (13) gave two different interpretations of the contents of this sieve tube. On page 296 he stated that the spindle-shaped bodies depicted in the sieve tube were probably leucoplasts. On page 300 he said that in this sieve tube spindle-shaped masses of slime were retained to the end (meaning through maturity). In our estimation the second interpretation is the correct one. Our observations have indicated that the occurrence of dispersal or nondispersal can be related to the position of the sieve elements relative to the vascular bundles. In the sieve elements within the vascular bundle the P-protein bodies normally disperse (Fig. 1); in the extrafascicular sieve elements the bodies often fail to disperse (Fig. 1, arrows; Fig. 2).

The distribution of the P protein in the mature sieve element is, of course, related to the phenomenon of dispersal or nondispersal of the P-protein bodies. In particular, the fate of these bodies affects the relation of the P protein to the mature sieve plate. Details of the dispersal of the P-protein bodies can be followed at the electron microscope level. The P-protein bodies first swell until they almost fill the lumen of the sieve element. Various cell organelles are seen trapped between the swelling P-protein bodies during this process (Figs. 3 and 4). About this time the changes characteristic of maturation of the sieve elements take place; the breakdown of the nucleus

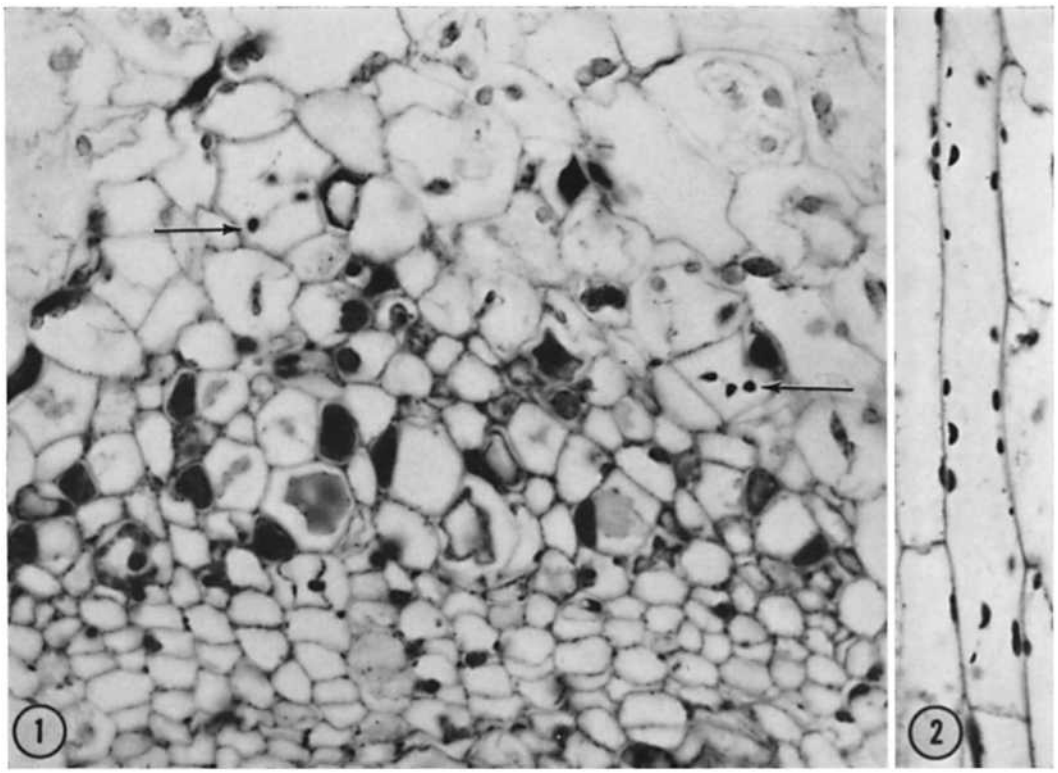


FIGURE 1 *Cucurbita maxima.* Light micrograph of a portion of a cross-section of a stem showing the phloem elements in the vascular bundle and also some extrafascicular sieve elements. At the arrows are mature extrafascicular sieve tubes with nondispersed P-protein bodies. × 640.

FIGURE 2 *Cucurbita maxima.* Light micrograph of a longitudinal section through extrafascicular sieve elements from stem. Numerous nondispersed P-protein bodies are evident. × 640.

and tonoplast is initiated, the ribosomes and dictyosomes begin to disappear, and the endoplasmic reticulum is reorganized (7). Fig. 3 shows a cross-section of a sieve element in which the P-protein bodies are beginning to disperse. The four large P-protein bodies have swelled and almost fill the cross-sectional area of the cell. The P-protein component in these bodies is of the fibrous P3 type (4). The cytoplasm between the P-protein bodies contains numerous ribosomes, dictyosomes, endoplasmic reticulum cisternae, and mitochondria. Some plastids are also evident in the cell. A longitudinal view of a cell at the same stage of differentiation, or perhaps at a slightly later stage, is illustrated in Fig. 4. Two of the bodies have fused, and the cytoplasmic components have become largely restricted to the parietal position. The cell has a nacreous wall. The cytoplasmic components other than the plastids, the mitochondria, the P protein, and the reorganized endoplasmic reticulum disappear, and the P-protein material becomes dispersed throughout the lumen of the maturing sieve element.

With the maturation of the protoplast the sieve plate becomes perforated. The appearance of mature sieve plates in electron micrographs is determined both by the preparation procedures for electron microscopy (especially the cutting and release of pressure in the sieve elements) and by the condition of the sieve-element contents, notably that of the P-protein components. In a sieve element in which a complete dispersal of the bodies has taken place the P-protein material tends to block the sieve-plate pores and may aggregate in longitudinally oriented strands leading from the pores (Fig. 5). In sieve elements in which the P-protein bodies retain their discrete

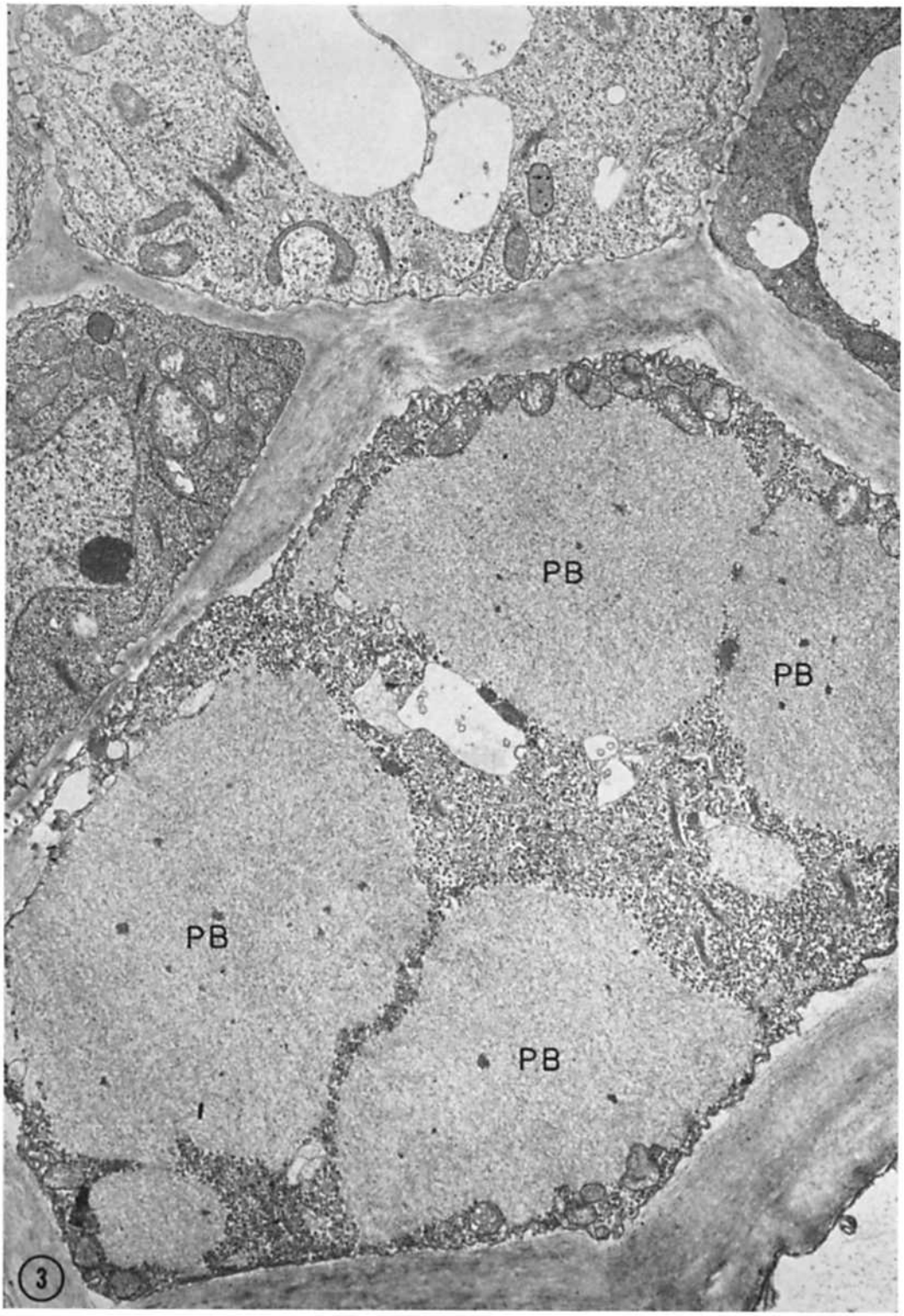


FIGURE 3 *Cucurbita maxima.* Transverse section of a sieve element and adjacent cells showing five P-protein bodies (*PB*) which almost fill the cross-sectional area of the cell. × 12,000.

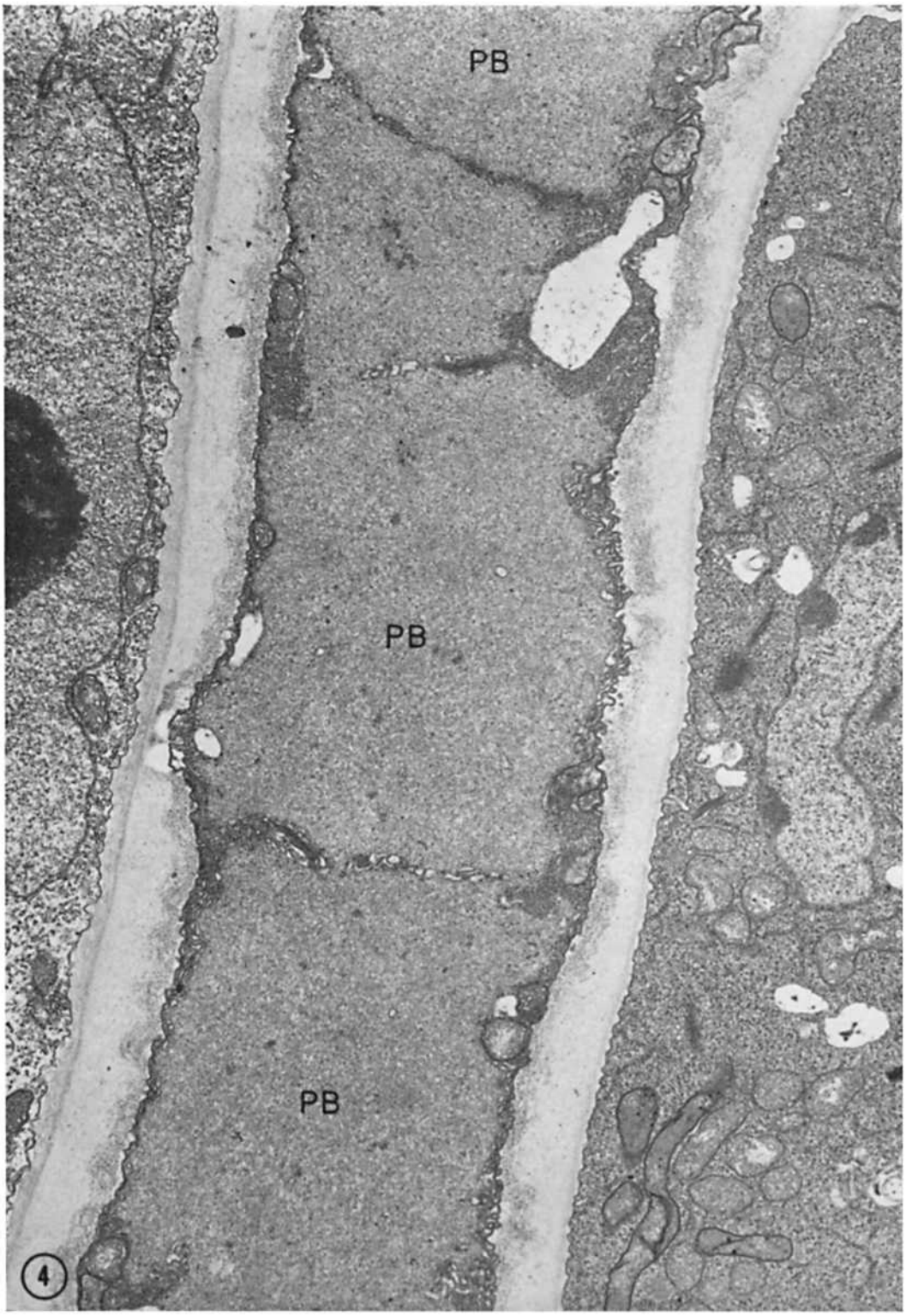


FIGURE 4 *Cucurbita maxima.* Longitudinal section showing a portion of a sieve element and adjacent cells. The P-protein bodies (*PB*) are swollen and are fusing. Cytoplasmic components are trapped between the P-protein bodies. × 9,500.

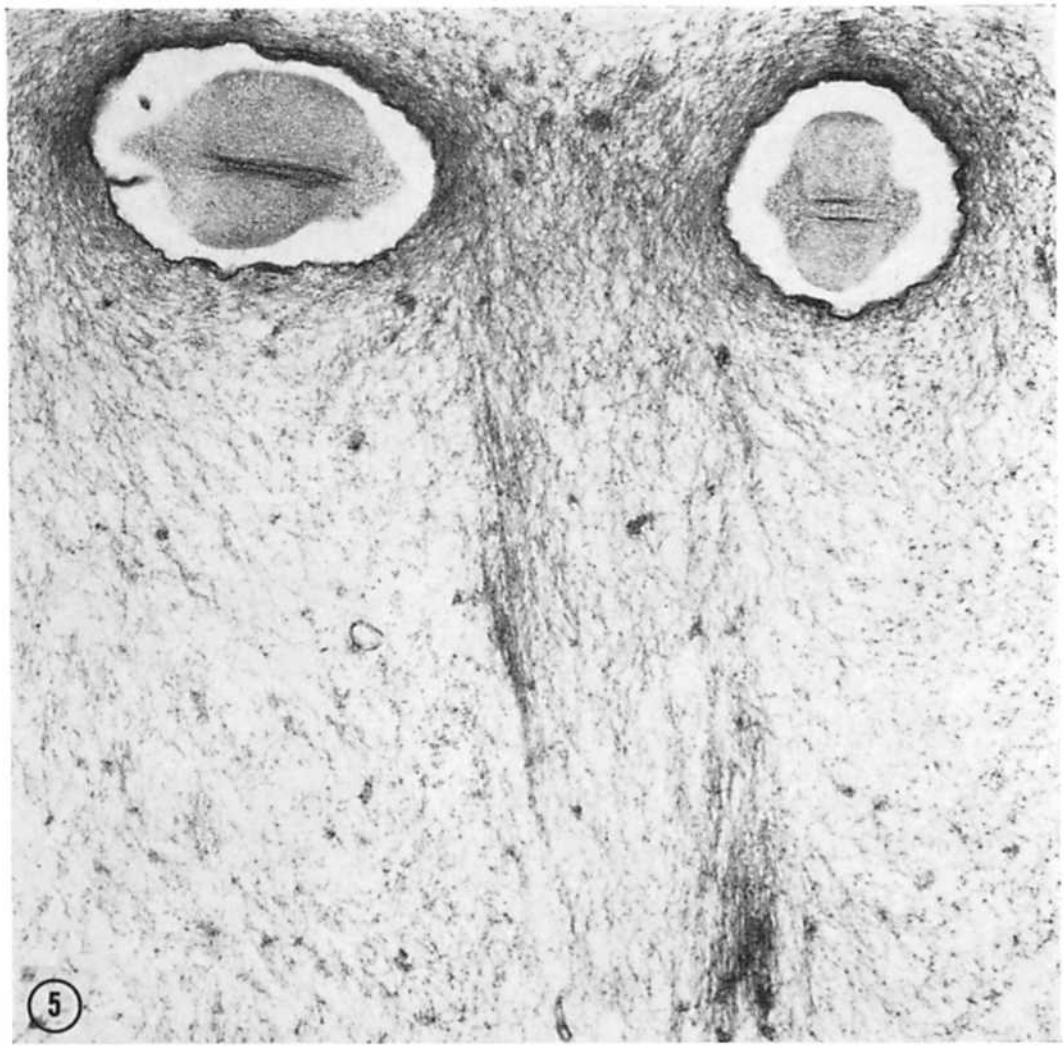


FIGURE 5 *Cucurbita maxima.* Longitudinal section of a portion of a sieve element and sieve plate. The P-protein material is loosely aggregated into a strand leading from the sieve-plate pore. × 27,000.

form, a small amount of dispersed P-protein material is frequently present. Possibly the degree of dispersal of the P-protein bodies is a function of individual cells. Fig. 6 shows a portion of a mature sieve tube and includes a sieve plate. Two dense, nondispersed P-protein bodies are evident in the upper sieve element. Some dispersed P protein is present; its accumulation on one side of the sieve plate is greater than on the other. The pores are relatively unobstructed by P protein. Little callose occurs around the sieve-plate pores. Fig. 7 shows another portion of a mature sieve tube including a sieve plate and a nondispersed P-protein body. In this micrograph, the P-protein body is not so electron opaque as are the bodies observed in Fig. 6. The P protein appears less compact than it does in the bodies in Fig. 6, and some material seems to be breaking away from the periphery of the body. In this view also, the sieve-plate pores are relatively unplugged although P-protein material is present in the pores and at the side of the sieve plate. A small amount of callose occurs around the sieve-plate pores. The sieve elements presented in Figs. 6 and 7 were judged to be extrafascicular sieve elements located next

FIGURE 6 *Cucurbita maxima.* Longitudinal section of a portion of a sieve tube including a sieve plate. The sieve tube was judged as extrafascicular in view of the large diameter, small amount of callose, and presence of nondispersing P-protein bodies (*PB*). The sieve-plate pores appear relatively unobstructed. × 6,750.

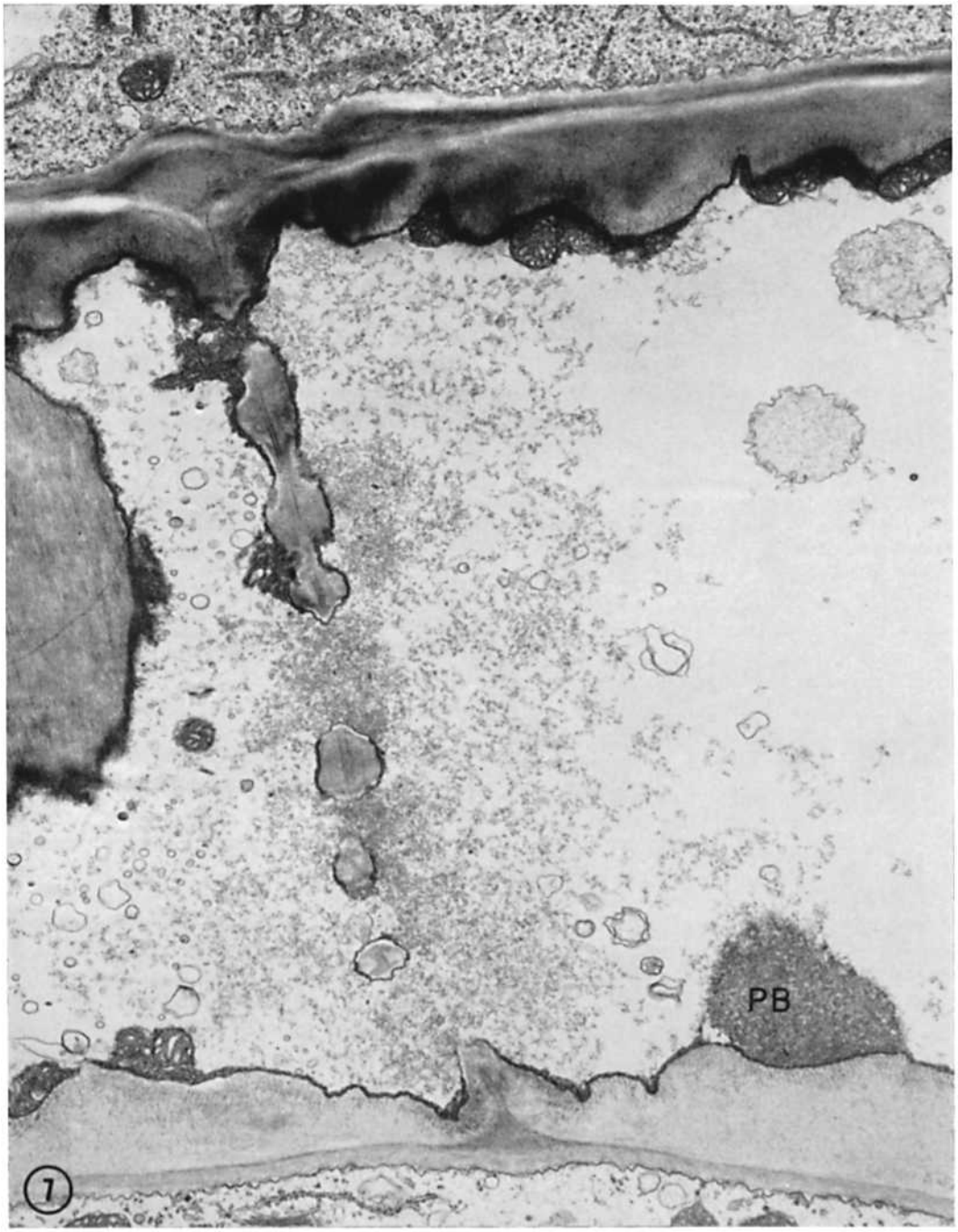


FIGURE 7 *Cucurbita maxima.* Longitudinal section of a portion of a sieve tube including a sieve plate. The sieve tube was judged as an extrafascicular one. The sieve plate has a small amount of callose, and the pores are relatively unobstructed. A nondispersed P-protein body (*PB*) is present. The nondispersed P-protein body is more loosely organized than those shown in Fig. 7, and P-protein material is breaking away from its surface. × 10,400.

to the phloem of a bundle, although the exact position was not unequivocally determined.

The preceding discussion has dealt with the large type of P-protein body whose components are fibrils (P3 protein) or narrow tubules (P4 protein). As was detailed in the earlier paper (4), smaller bodies consisting of P1-protein tubules also occur in *Cucurbita* sieve elements. These bodies frequently seem to fail to disperse in both the fascicular and the extrafascicular sieve elements. Fig. 12 shows an undispersed P1-protein body in a sieve element apparently derived from the extrafascicular system.

The variations in the relation between the P protein and the sieve plates depend not only on the dispersal and nondispersal of the P-protein bodies; the accumulations on the plates and in the pores vary also in the sieve elements in which P-protein bodies disperse. Figs. 8–12 illustrate some of the variations in these relations. It may be assumed that Fig. 12 exemplifies the typical unblocked condition in an extrafascicular sieve element. The other four sieve plates are from cells with dispersed P protein. The accumulations of the P protein vary in density on the plates and in the pores; and the pores may be relatively unplugged despite the accumulations on the plate (Fig. 9), or on the contrary, the pores are plugged in the absence of heavy accumulations on the sieve plate (Fig. 10). Fig. 11 illustrates the typical plugging of pores in the presence of a dense accumulation on one side of the sieve plate (slime plug). It is generally accepted that P-protein accumulations on the sieve plates result from a differential release of pressure in the severed sieve tubes, but the variations in the form of these accumulations as depicted in Figs. 8–11 are difficult to explain. One may assume that environmental conditions, such as proximity to the cut, are involved, but there may be also internal conditions in individual cells that affect the disposition of the disturbed contents. A good illustration is provided by the extrafascicular sieve elements in which the sieve plate may remain unplugged or may be blocked by the P-protein bodies themselves which appear to become dislodged toward the sieve plate from their usual parietal position. In Figs. 8–11 callose is present in variable amounts and cannot be related to the degree of blocking of the pores with the P protein. Most of the pores in Fig. 8 show no dense plugs despite large amounts of callose. In Fig. 9, two pores are filled with P protein, and the third is not, although the amount of callose is the same in all pores. In Fig. 10 the plugging is dense even though the callose lining of the pores is thinner than that in the relatively unplugged pores in Fig. 8. Thus the blocking of the pores by P protein cannot be explained in terms of a compaction of P-protein material within the pores induced by callose deposition on fixation, as has been suggested by Northcote and Wooding (11).

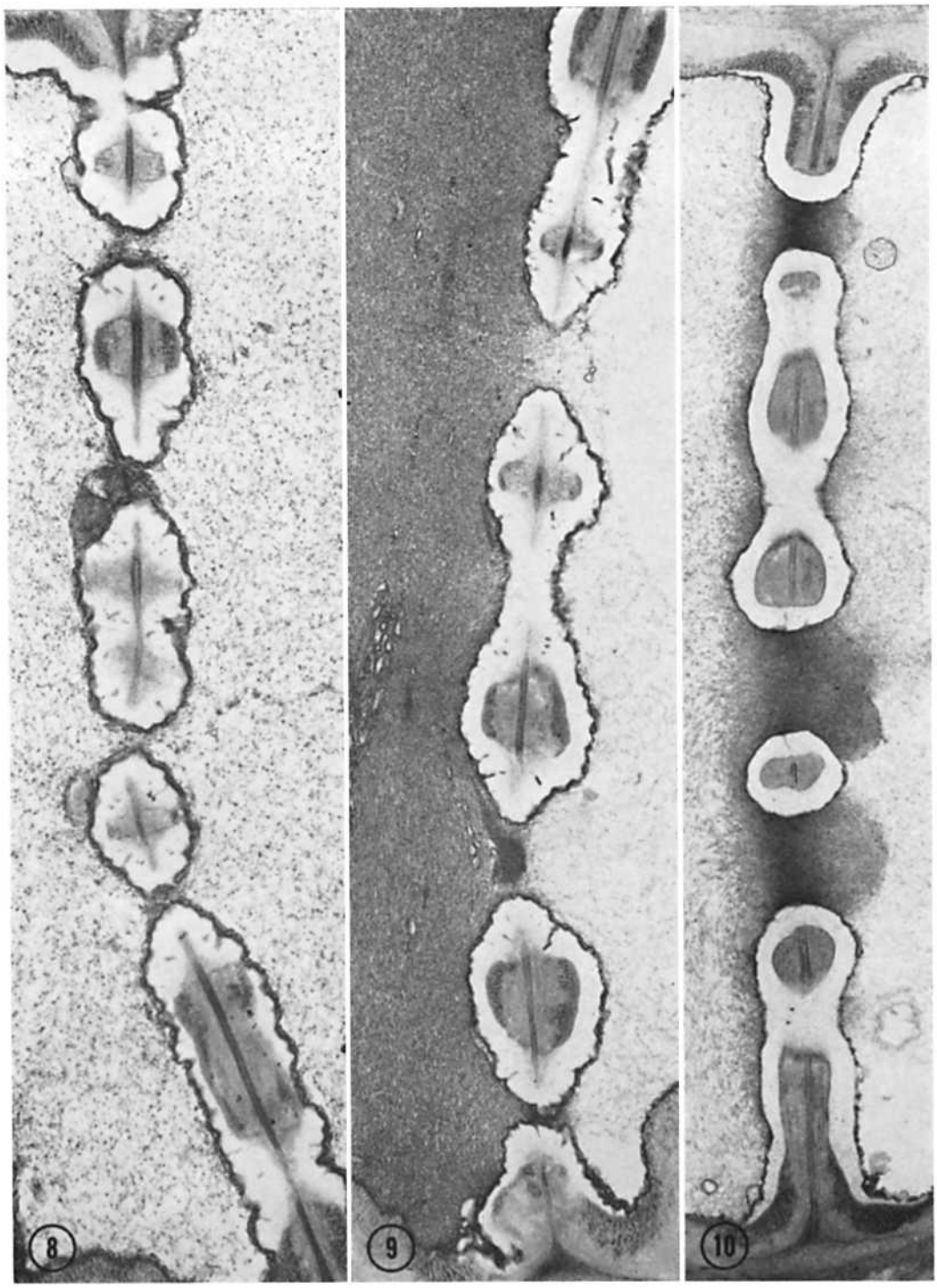
Some form of strand formation can frequently be discerned in the P-protein material associated with the sieve plates (Figs. 5, 11). These strands do not always run in the longitudinal direction within the sieve elements. Sometimes the orientation of the P-protein components is in the transverse direction of the slime plugs (Fig. 9).

The nondispersing slime bodies characteristic of the extrafascicular sieve elements are usually closely appressed to the plasma membrane. In several cases we have observed microtubules tightly packed between the P-protein bodies and the plasma membrane. This observation is recorded in Fig. 13.

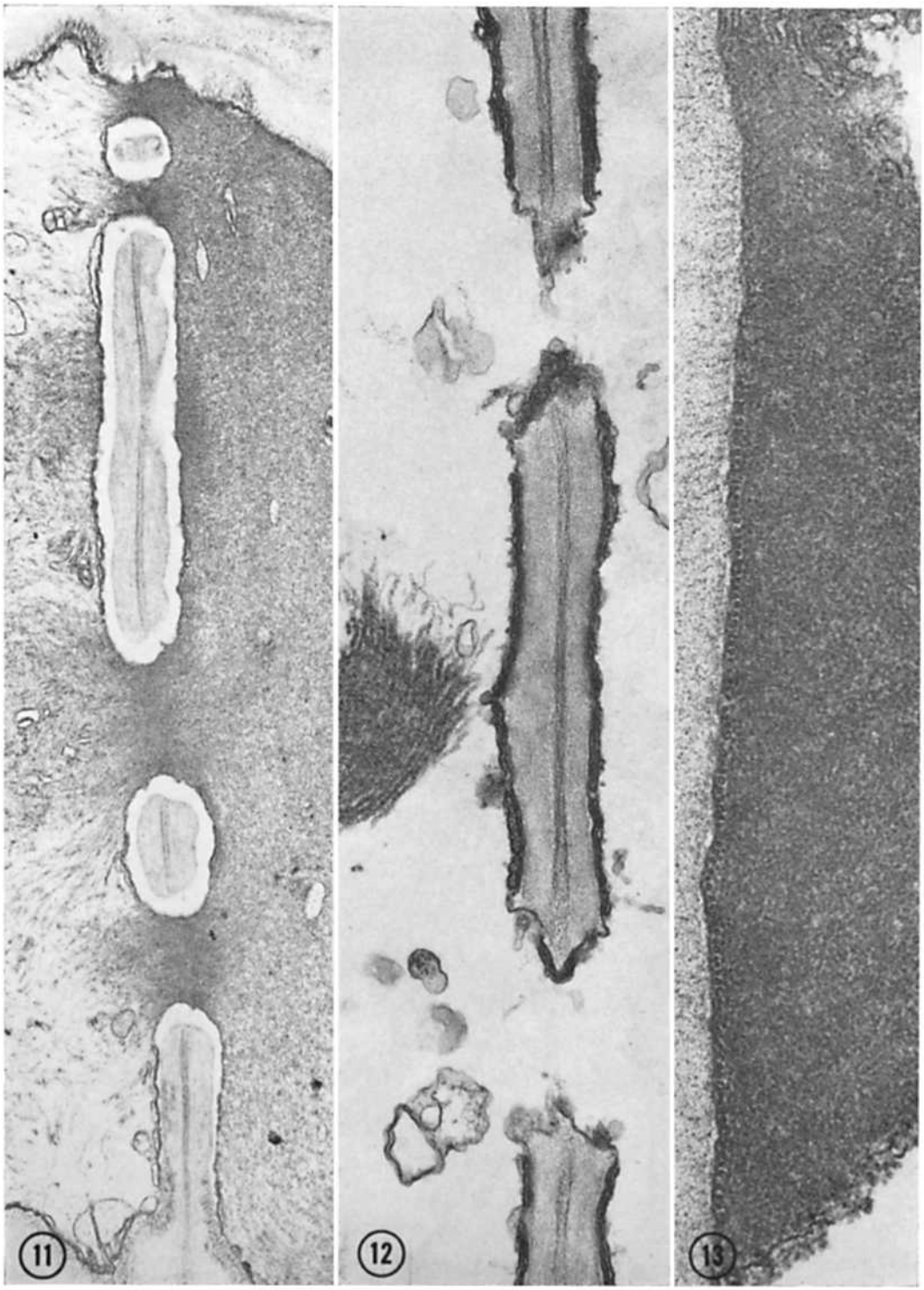
DISCUSSION

Although in some sieve elements the P-protein bodies fail to undergo the swelling and dispersal phenomena, slime-plug formation is possible in such cells. The bodies themselves may be displaced toward the sieve plates. This particular phenomenon does not strictly separate the sieve elements with dispersing and nondispersing P-protein bodies. The displacement of P-protein bodies toward the sieve plates may occur in sieve elements with the usual ontogeny of the P-protein bodies (e.g., see references 2, 8). The dispersal or nondispersal of the P-protein bodies can be related to the position of the sieve elements in the complex phloem system of *Cucurbita*. Within the vascular bundles, P-protein bodies of the sieve elements normally disperse. In the extrafascicular system, the P-protein bodies often fail to disperse. This phenomenon is yet to be related to the observations of Fischer (9) and Crafts (2) that the extrafascicular sieve elements eventually become completely filled with granular or highly refractive material.

At the electron microscope level, the morphological association of the sieve plate and P-protein components is in part determined by the degree of dispersal of the P-protein bodies. One extreme condition is found in sieve elements of the extrafascicular system in which the P-protein bodies do



FIGURES 8–10 *Cucurbita maxima.* Longitudinal sections through sieve plates to show the variety of images obtained from various sieve tubes. Fig. 8, sieve tube with evenly dispersed P protein and most sieve-plate pores relatively free of the component. Fig. 9, sieve tube with a P-protein plug (slime plug) which has a transverse orientation of the fibrillar P-protein component. One sieve-plate pore is open. Fig. 10, sieve tube in which there is a much greater accumulation in the sieve-plate pores than in the lumen of the cells. The sieve plates shown in Figs. 8, 9, and 10 have a moderate amount of callose. Fig. 8, × 16,500; Fig. 9, × 10,800; Fig. 10, × 9,600.



FIGURES 11 and 12 *Cucurbita maxima.* Longitudinal sections through sieve plates to show the association of the sieve-plate pores and the P-protein component. Fig. 11, a dense accumulation of P protein at one side of the sieve plate extends through the pores. A moderate amount of callose is present. Fig. 12, sieve plate apparently without callose deposit, and the pores are unfilled. A P-protein body of the small tubular type is near the sieve plate. Fig. 11, × 13,600; Fig. 12, × 35,000.

FIGURE 13 *Cucurbita maxima.* Section of a portion of a nondispersed P-protein body adjacent to the plasma membrane. Between the P-protein body and the plasma membrane is a row of microtubules. × 51,000.

not disperse, the sieve-plate pores are relatively unobstructed, and little callose surrounds the pores; the other extreme is represented by the sieve elements within the vascular bundle which have dispersing P-protein bodies, pores filled with a P-protein component, and are usually surrounded by large cylinders of callose. Between these extremes are intermediate conditions characterized by partially dispersed P-protein bodies. The question, of course, arises whether the sieve elements showing the various conditions of the sieve plates are all functional in long-distance transport of nutrients. The cytological identification of functional sieve elements at the light and the electron microscope levels continues to be uncertain. The phenomenon of the filling of the sieve-plate pores with P protein is obviously of fundamental importance in any consideration of phloem transport; this aspect has been discussed in a previous report (3).

Aggregations of P-protein fibrils can assume the form of strands. This phenomenon is most evident in the regions of the sieve-plate pores, and at the electron microscope level, strands can be seen leading away from the pores for some distance. These strands obviously correspond to those which Crafts (2) observed with the light microscope and interpreted as being cytoplasmic strands, and as pointed out by Northcote and Wooding (11), they also constitute the transcellular strands of Thaine (see reference 14). Evert et al. (8) correctly identified the strands as aggregates of P-protein fibrils, but they postulated that these strands were continuous from element to element through the sieve plate and existed in the intact plant. Aggregation of P protein can also take place in such a way that the strands are oriented in the transverse direction (Fig. 9). This form of aggregation suggests that the strandlike condition of the P protein is an artifact appearing in response to injury resulting from cutting of the phloem conduit. The more common vertical orientation of the strands probably reflects the direction of flow caused by the release of pressure in the severed sieve tube.

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