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Anatomical Aspects of Abscission

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Abstract. **Anatomical aspects of abscission are reviewed mainly on the basis of experimental studies on *Coleus, Gossypium,* and *Phaseolus.* In *Phaseolus* histological studies of explants show that petiolar abscission is correlated with the formation of tyloses in the vessels proximal to the zone of separation, and that the abscission zone is much less well demarcated than in *Coleus* or *Gossypium.* Radioautographic studies of *Phaseolus* petiole explants indicate little initial difference in the distribution of nucleolar RNA and in nuclear and cytoplasmic protein in cells distal (toward the blade) or proximal (toward the stem) to the region of separation. However, in ethylene-treated explants an increase in nucleolar RNA and in nuclear and cytoplasmic protein is evident in cortical cells immediately proximal to the abscission zone, and binucleate cells commonly occur. Abscission occurs by dissolution of newly formed cell walls and disruption of the mother cell walls in the zone of recently divided cells. It is suggested that the experimental results can be explained on the basis of changes induced in levels of ethylene in the petiole, the experimental application of ethylene becoming effective in expediting abscission only after the endogenous ethylene level in explants has declined.**

This morning I shall attempt to beguile you with a few of the many-splendored facets of the anatomy of leaf abscission. From the studies of Lee (22), Lloyd (28), Pfeiffer (33), Sampson (35), Tison (42) and many others have come extensive descriptions of anatomical changes during abscission. It is not within the realm of this seminar to review that information in detail, but rather to bring together some of the various physiological and biochemical data which have accrued and which bear upon the broad subject of the anatomy of leaf abscission. These data have been garnered mainly from experimental studies on the induction, acceleration, and retardation of leaf abscission by diverse endogenous and exogenous substances, particularly in *Gossypium, Coleus,* and *Phaseolus* (4, 18, 34). My remarks this morning will deal largely with the morphological basis of leaf abscission in these 3 plants.

During the past year we have been involved with a series of anatomical and histochemical analyses of ethylene-treated explants of *Phaseolus vulgaris* L. cv. 'Red Kidney'. To accurately interpret the results of studies on these explants and to justify the approach we have taken, the following observations are particularly relevant:

(1) Not all explants in a given sample abscise at the same time; in fact, separation is usually characterized by the time to 50 % abscission. A range of anatomical changes may thus be encountered among explants at any given experimental time, and so the establishment of strict anatomical timetables is necessarily arbitrary. Large numbers of explants must be examined before any patterns of change can be considered typical. The fact that anatomical variation exists within a sample of *Phaseolus* explants is not particularly surprising since in the intact plant abscission of oppositely placed primary leaves proceeds at very different rates. In this connection it is of interest that oppositely placed axillary buds are of different sizes (fig 1). No correlation has been demonstrated between the time of leaf fall and the bud size; a correlation may exist, however, between the rapidity with which some of the early, visible changes leading to abscission occur (*i.e.*, loss of chlorophyll, loss of turgor) and the size of the bud.

(2) Abscission zones frequently differ from contiguous regions in having a minimum of strengthening tissue and have been characterized as zones of weakness in mature leaves of woody plants (3, 22, 36). In *Phaseolus* the region of separation does correspond with a zone of hyaline cells in the outer cortex of the petiole and such cells are frequently associated with a region of weakness. But, more importantly, the separation region in *Phaseolus* is abruptly set off by a lack of sclerification in the cells of the pith (fig 2) and by the characteristic short, broad configuration of the tracheary elements (fig 3) (15). Separation occurs at the juncture of the pulvinus and petiole, where the single, amphicribral bundle of the pulvinus diverges to form the 6 to 8 leaf traces of the petiole. On the basis of such features as these, perhaps the region of separation of *Phaseolus* could be interpreted as being structurally weak, but it seems more accurate and more to the point to regard it as primarily a region of abrupt structural transition.

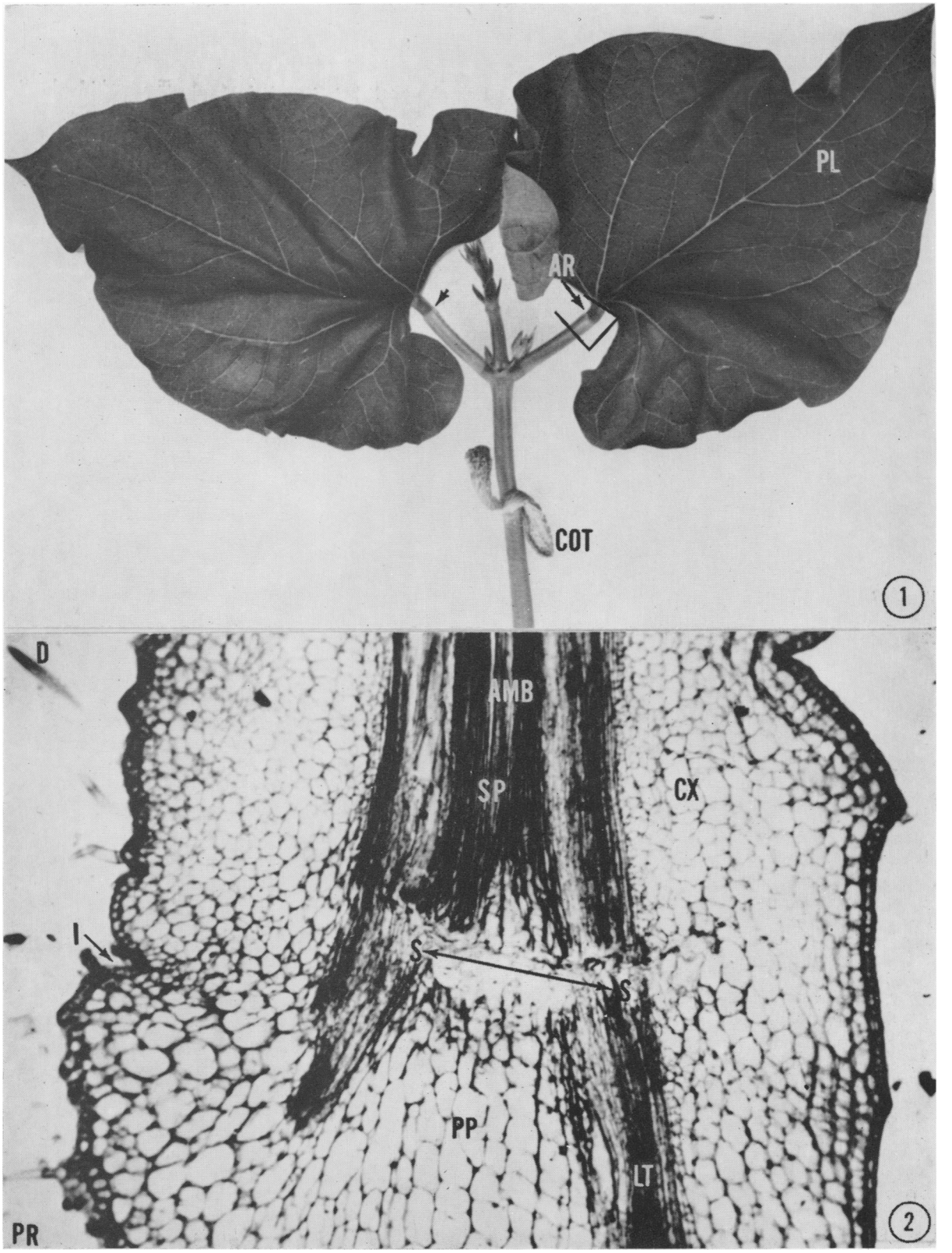
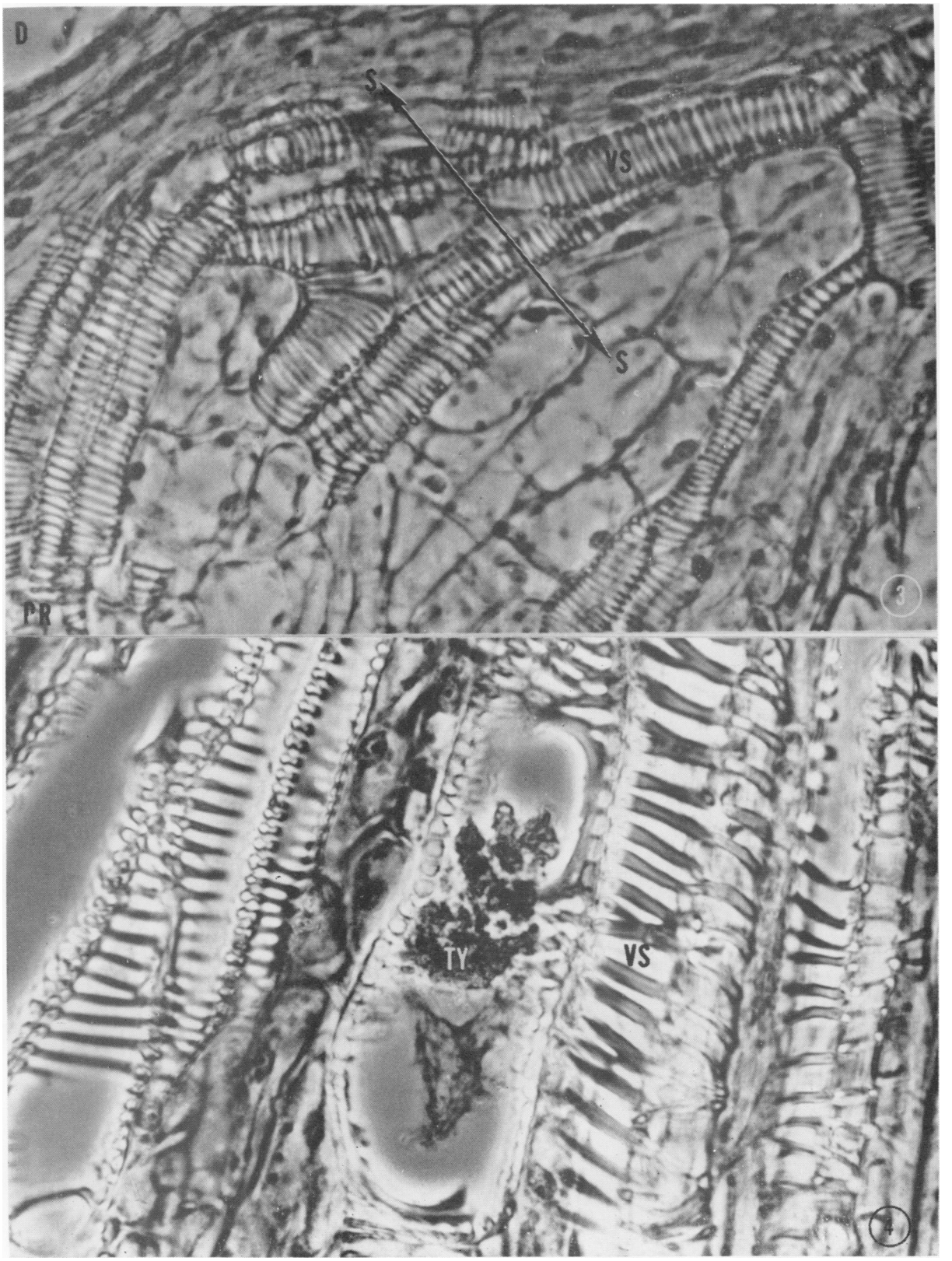


FIG. 1. Fourteen day old plant of *Phaseolus vulgaris*, L., cv. 'Red Kidney,' with fully expanded primary leaves, showing the location of the laminar abscission region (arrows) and the extent of the explant (bracket). Note also the unequal size of the axillary buds. ×1.

FIG. 2. Photomicrograph of a longitudinal section of part of a bean explant, showing the region of separation at the juncture of the pulvinus and the petiole, and some of the structural differences between the distal and proximal parts of the explant. ×90. (From H. S. Brown and F. T. Addicott, 1950.)



FIGS. 3-4. Characteristics of the abscission region of the bean. Figure 3) The short, broad configuration of the xylem elements at the juncture of the pulvinus and the petiole. ×1300. Figure 4) The occurrence of tyloses in the vessel elements. ×2600.

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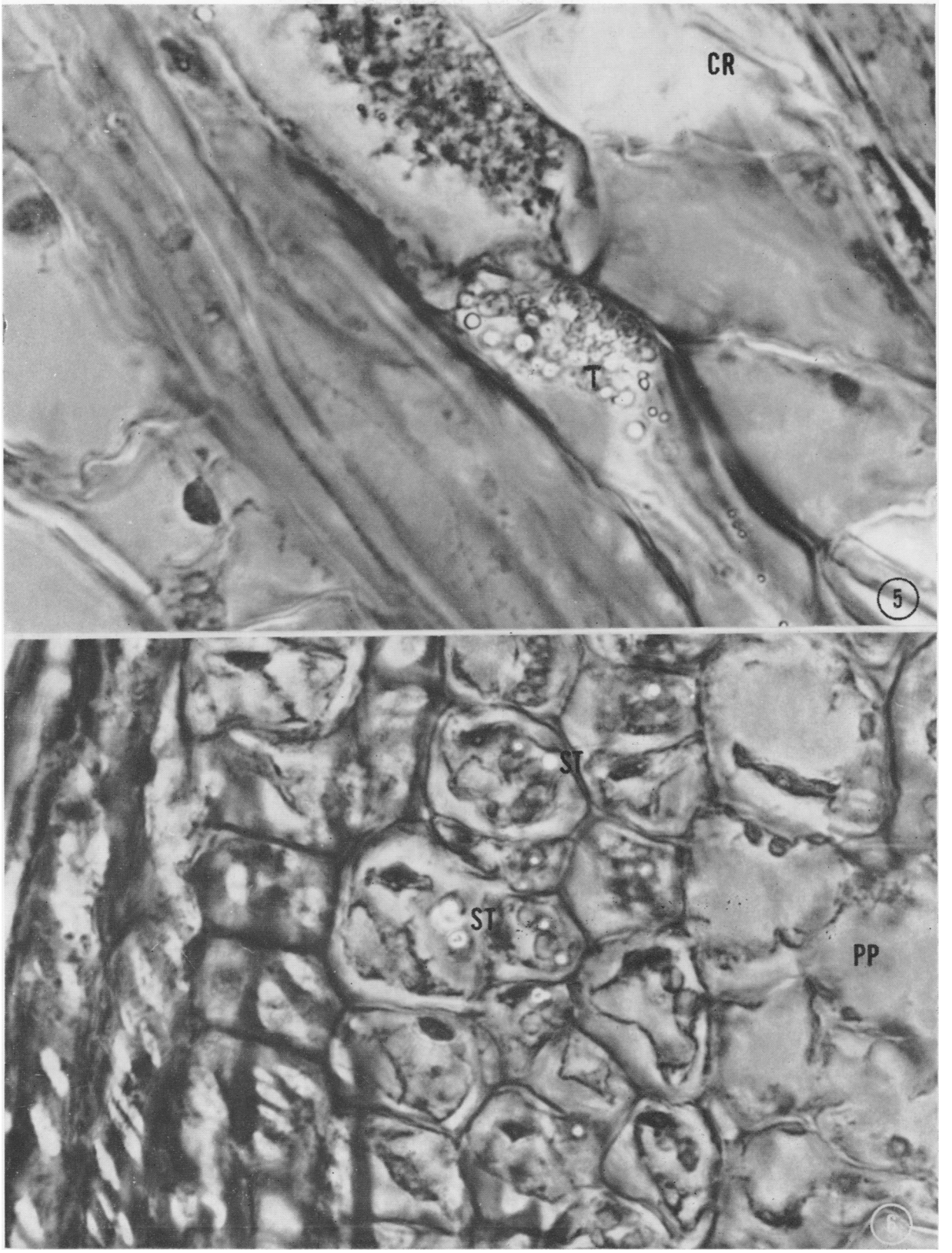

FIGS. 5-6. Characteristics of the abscission region of the bean. Figure 5) Tannin-like inclusions in the phloem cells. Note also the presence of crystals in the phloem parenchyma. ×3200. Figure 6) Starch-filled cells of the sheath which surrounds the vascular bundles. ×3200.

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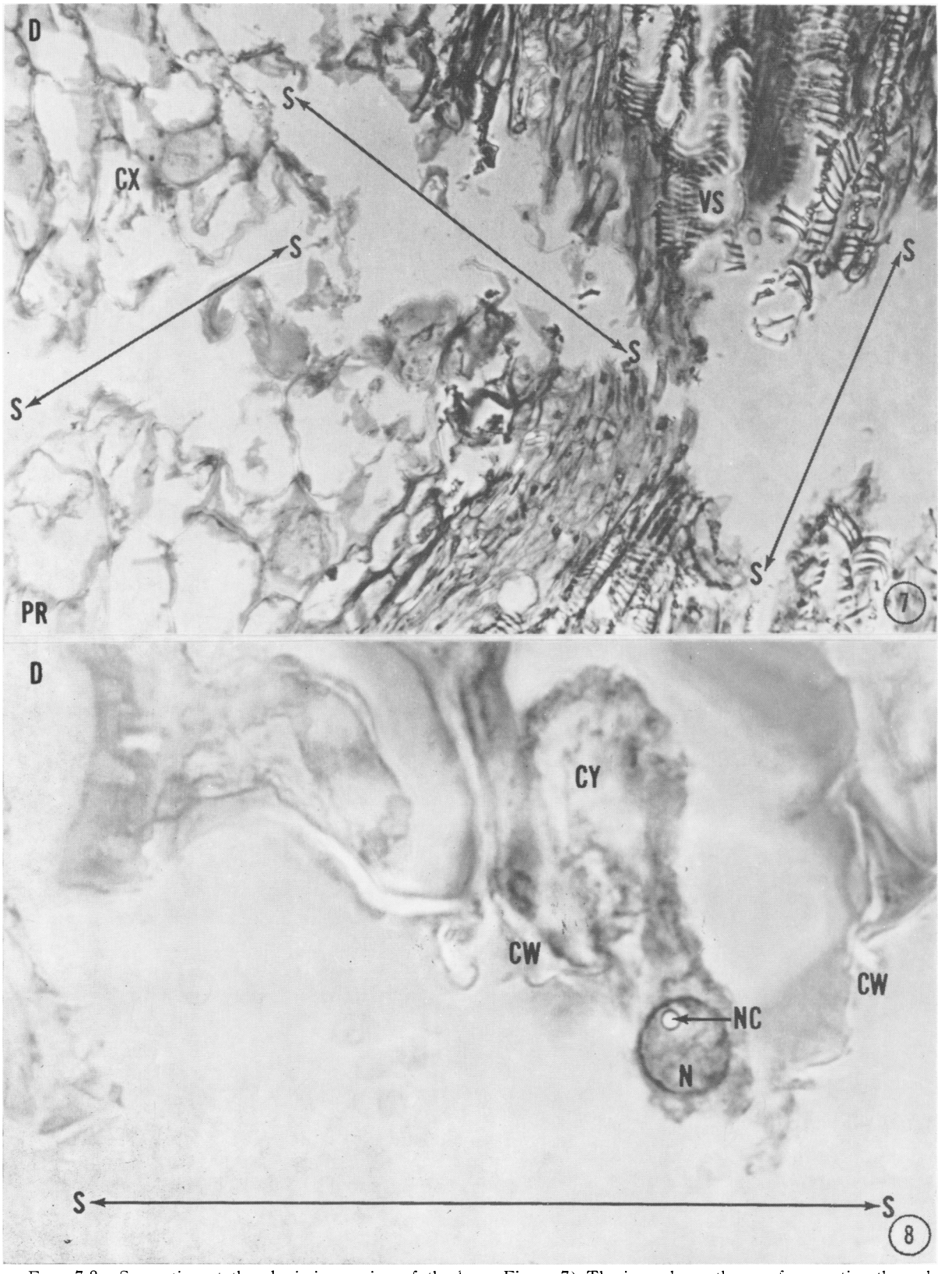

FIGS. 7-8. Separation at the abscission region of the bean. Figure 7) The irregular pathway of separation through the cortex and vascular tissue. ×100. Figure 8) Separation by breakdown of both the middle lamella and the cell wall, and extrusion of cell contents into the separation cavity. ×3200.

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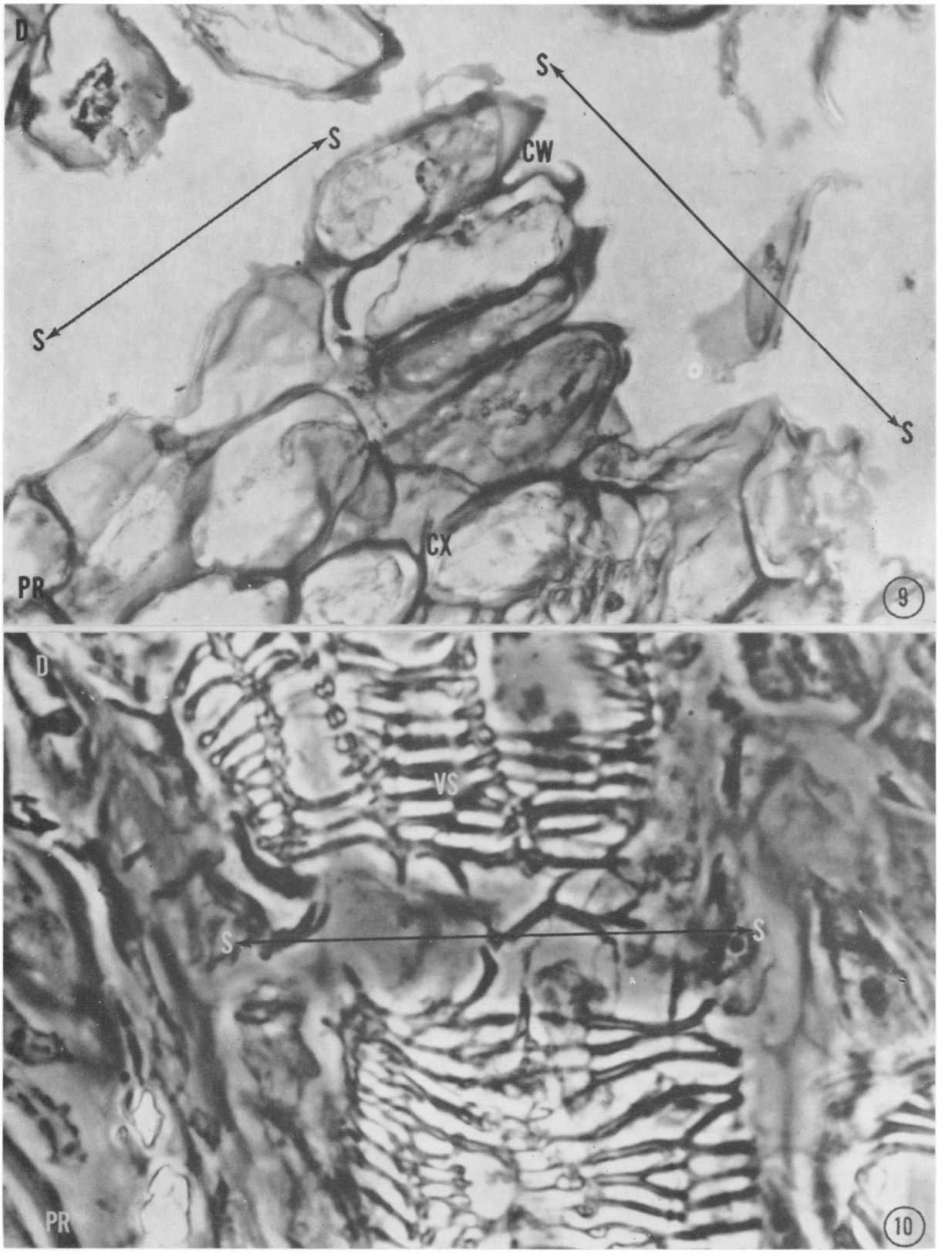

FIGS. 9-10. Separation at the abscission region of the bean. Figure 9) Separation by dissolution of the middle lamella only, walls and contents of cells abutting the separation cavity remaining intact. ×2600. Figure 10) Separation by mechanical breakage of the vessel elements. ×2600.

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(3) The changes which certain cell inclusions undergo as abscission proceeds are unique to the abscission zone. For example, as explants age, vessels proximal to the region of separation frequently become occluded with tyloses (fig 4) (11, 22, 31). Scott *et al.* (39) noted that tylose formation in *Phaseolus* could be directly correlated with abscission; Bornman (8) was unable to establish such a causality in *Gossypium*. Phloem elements in the region of separation in *Phaseolus* contain callose (38) and tannin-filled elements (fig 5), callose will diminish in amount as abscission proceeds. Starch also occurs, in the cortical parenchyma and in cells of the sheath which surrounds the vascular bundles (fig 6). An increase in starch synthesis in cortical cells during the first 24 hours following excision of *Phaseolus* explants may be correlated with a concomitant increase in number of cells in that region. Similar changes in starch synthesis during abscission have been noted in *Gossypium* explants (9).

(4) The abscission zone and the separation layer in *Phaseolus* cannot be as precisely delimited histologically as in *Gossypium* (10) or *Coleus* (43). In *Phaseolus,* separation follows an irregular and tortuous pathway through the cortex, vascular tissue, and pith (fig 7). Consequently, slicing through the explant to analyze changes in proximal and distal segments may give questionable results, particularly in regard to changes in cells close to the separation region.

Separation in *Phaseolus,* as well as in many other plants, may occur in 3 ways (15, 22, 33): first, by dissolution of the middle lamella and primary wall, after which the intact nucleus and cytoplasm are frequently extruded into the separation cavity (fig 8); second, by dissolution of the middle lamella only, in which case cells which separate remain intact (fig 9); and third, by mechanical breakage, which involves sieve elements, tracheary elements and other nonliving cells (fig 10).

These general structural characteristics of the abscission region, plus a consideration of some current physiological concepts of leaf abscission, have formed the basis for this anatomical approach.

Materials and Methods

One of the more promising contemporary hypotheses of the mechanism of abscission has been proposed by Abeles and Holm (1). They have suggested that applied ethylene acts as an effector substance to stimulate RNA synthesis and protein synthesis in the separation region of *Phaseolus* explants. Such a concept is particularly interesting in view of current studies on mobilization and senescence in abscission (24, 26, 30, 32, 37), and it points up the pertinence and the importance of determining the exact localization of histochemical changes in the abscission zone. We have observed differences in the location and distribution of RNA and protein in the abscission region of ethylene-treated explants of *Phaseolus* by the preparation of radioautographs, following incorporation of labeled uridine and leucine, and by histochemical staining.

Plant Material. Bean plants were grown for 14 days in vermiculite at 25°, with 2000 ft-c of light given for 16 hours per day. Explants were cut to include the laminar abscission region of the primary leaf (fig 1), and comprised 0.5 cm of pulvinal (distal) tissue and 0.5 cm of petiolar (proximal) tissue (2); the region of separation lay midway between the distal and proximal parts, and was discernable externally as a narrow, slightly constricted band of hyaline cells.

Histological Techniques for RNA Analysis. For studies of RNA distribution, uridine-^{3}H was introduced at the severed base of the petiole of the primary leaf at a concentration of 1 μc per ml in half strength Knop's solution. (This did not alter the subsequent rate of abscission.) After 1 hour explants were cut, inserted proximally in 1 % agar for 24 hours, following which they were treated with 10 μl/liter ethylene for 4 and for 8 hours. Fixation for anatomical study was in cold formalin-acetic acid-alcohol (FAA); dehydration was through the standard tertiary butyl alcohol (TBA) series (20), and after embedding in Tissuemat the explants were sectioned at 6 μ. For the preparation of radioautographs, mounted sections were coated with Ilford K_2 liquid emulsion and refrigerated in darkness for 2 to 6 weeks. Slides were developed in Kodak D 19, fixed and washed for 1 hour. Using the techniques described by Tepper and Gifford (40), radioautographs were stained with 2 % aqueous pyronin Y, which is specific for RNA; DNA does not stain. Pyronin Y does, however, stain nucleoli, heterochromatic regions of interphase nuclei, entire chromosomes during mitosis, spindle apparatus, cytoplasm, hila of starch grains, and thick secondary walls of xylem elements. When sections are placed in a ribonuclease solution containing 0.25 mg/ml of 0.05 M cysteine hydrochloride (at pH 6.8) for 3 hours at 55°, pyroninophilia is completely eliminated from all cell components other than the secondary wall and the hila of starch grains. RNA would not be expected to occur in the latter 2 structures; thus this procedure is followed to establish stain controls.

Under carefully controlled staining conditions, pyronin Y can be used to recognize patterns of distribution and qualitative differences in amounts of RNA, since its stain intensity ranges in color through shades of pink to red, increasing in intensity where increased amounts of RNA are localized.

Histological Techniques for Protein Analysis. Techniques similar to those described above were followed for incorporation of L-leucine-^{3}H into explants for observations of protein localization. Histochemical staining was with mercuric bromphenol blue, a general protein stain, which was used according to the procedures developed by Kunkel and Tiselius (21) and modified by Mazia *et al.* (29).

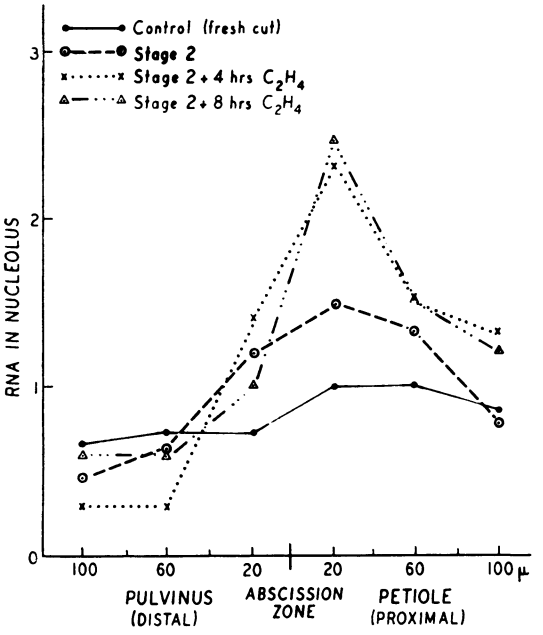


FIG. 11. The occurrence and distribution of nucleolar RNA in the region of abscission of the bean, as indicated by the staining intensity of pyronin Y.

Results and Discussion

Localization of RNA. Analysis of the distribution patterns of nuclear and nucleolar RNA were based on intensity of stain of pyronin Y, using numerical ratings of 1(very pale pink) to 3(bright red), and on the localization of silver grains representing labeled uridine, in cells in the abscission region. Results of the histochemical staining are presented in figure 11, and may be summarized by the following observations:

1) In freshly cut explants there was little difference in the distribution of nuclear or nucleolar RNA in cells distal or proximal to the region of separation. Nuclei and nucleoli were uniformly stained pale pink and scattered silver grains indicated that uridine-^{3}H localization was low (fig 12).

2) After explants were aged for 24 hours (Stage 2) and then treated for 4 or for 8 hours with ethylene, an increase in nuclear and nucleolar RNA content was apparent in cells immediately proximal to the abscission zone, *i.e.*, those which subtended the separation cavity. Nucleoli in such cells stained bright red, and silver grains denoting uridine-^{3}H incorporation were concentrated in the nuclei and nucleoli (fig 13).

3) The initial changes in apparent amount of nucleolar RNA occurred during the 24 hour aging period, and the patterns established during that time were maintained and accentuated by ethylene treatment.

Localization of Protein. Since protein distribution was determined by counting silver grains, isotope incorporation and histochemical staining were carried out separately. A summary of results of protein analysis using L-leucine-^{3}H is presented in figure 14. Counts of silver grains in cortical cells in a 200 μ^2 area at the abscission region indicated a fairly uniform distribution of nuclear and cytoplasmic proteins in freshly cut sections. Following ethylene treatment, counts of silver grains demonstrated a decrease in protein localization in distal cortical cells and an increase in protein localization in cortical cells at and immediately proximal to the abscission zone (figs 15, 16). These patterns of protein distribution shown by labeled leucine were borne out by histochemical staining with mercuric bromphenol blue. There was no noticeable difference in stain intensity between proximal and distal cells in freshly cut tissue, but after ethylene treatment nuclei in distal cells were barely stained and scarcely visible while those in cells proximal to the abscission region stained bright blue (figs 17, 18).

The changes in the apparent amount of RNA and of protein were not maintained in proximal cells further from the abscission zone. The increase in occurrence of RNA and of protein following treatment with ethylene was extremely localized, and involved only 2 to 6 tiers of cells.

The proposal of Abeles and Holm (1) that ethylene treatment effected RNA and protein synthesis rested largely on experiments which showed an enhanced incorporation of label (^{32}P and L-leucine-^{14}C) into RNA and protein in ethylene-treated explants (1, 17). Their data on the blockage of

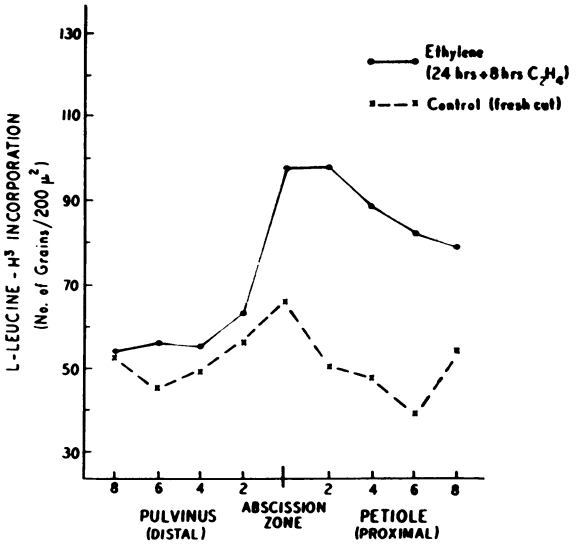
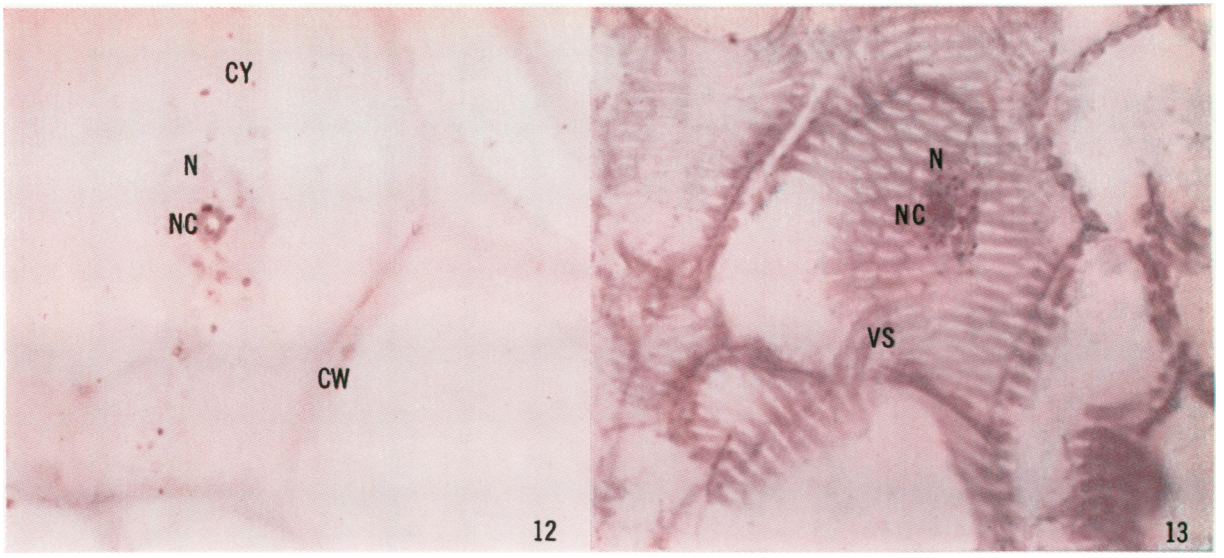
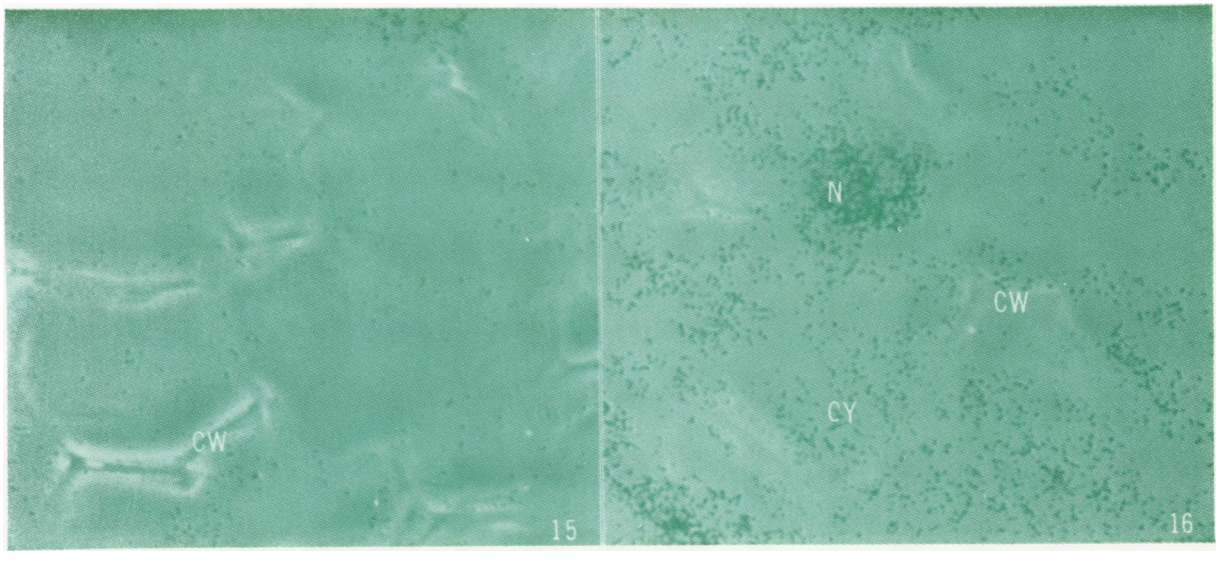


FIG. 14. The distribution of nuclear and cytoplasmic proteins in the abscission region of the bean as indicated by counts of the number of silver grains denoting the occurrence of L-leucine-^{3}H. (Based on counts compiled by Dr. Sanat Majumder and by the author.)



FIGS. 12-13. Radioautographs showing the occurrence of nuclear and nucleolar RNA in the abscission region of the bean, as indicated by histochemical staining with pyronin Y and incorporation of uridine-^{3}H. Figure 12) Cortical cells of a freshly cut explant showing scattered silver grains and pale pink staining. ×2600. Figure 13) Short, broad vessel element immediately proximal to the abscission region, after treatment of the explant with ethylene for 8 hours. Note the concentration of silver grains in the nucleus and nucleolus, and the bright red staining. Staining of secondary walls of xylem elements does not indicate the presence of RNA. ×2600.



FIGS. 15-16. Radioautographs showing the distribution of nuclear and cytoplasmic proteins in the abscission region of the bean, as indicated by labelling with L-leucine-^{3}H. Figure 15) The distribution of silver grains in cortical cells distal to the abscission region, after treatment of the explant with ethylene for 8 hours. ×2300. Figure 16) The distribution of silver grains in cortical cells immediately proximal to the abscission region, after treatment of the explant with ethylene for 8 hours. Note the increased number of silver grains in both the cytoplasm and the nucleus. ×2300.

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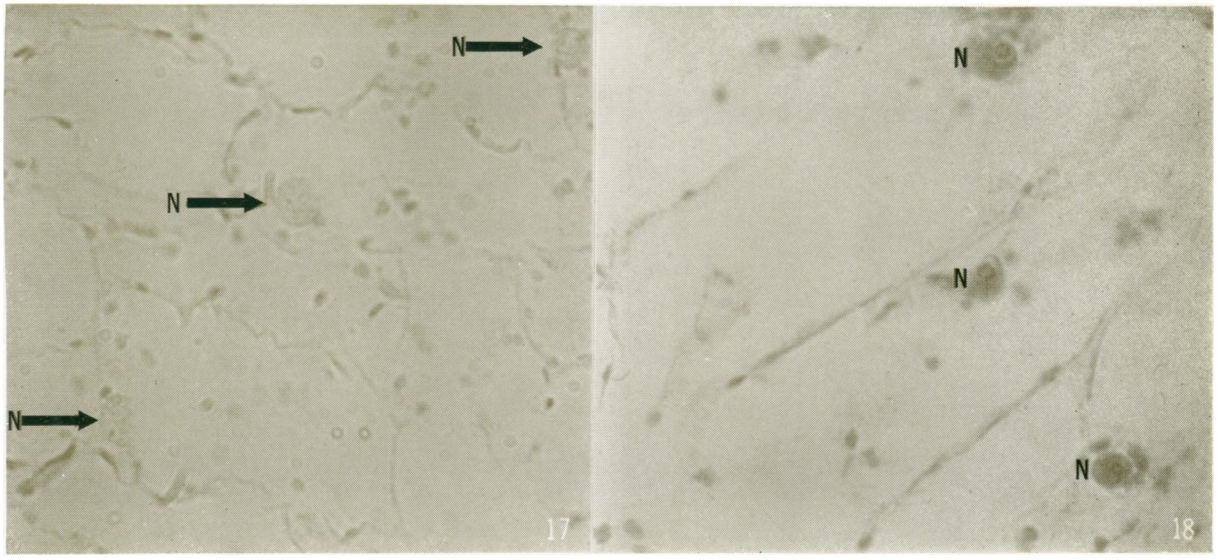

FIGS. 17-18. Histochemical staining with mercuric bromphenol blue for protein distribution in the region of abscission of the bean, after treatment with ethylene for 8 hours. Figure 17) Distal cortical cells, showing a pale blue staining of nuclei (arrows). ×2600. Figure 18) Proximal cells, showing an intense blue staining of nuclei. ×2600.

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abscission by inhibitors such as actinomycin D is always subject to the alternative interpretation that the inhibitor prevented synthetic activities necessary for abscission to progress. At the cellular level, we now have some evidence that ethylene treatment does, in fact, result in increased localization of RNA and of protein in a limited region of cells at and immediately proximal to the region of separation in *Phaseolus* explants. The fact that the enhanced localization of RNA is already apparent after the 24 hour aging period, before ethylene is applied, raises the interesting question of whether applied ethylene can properly be denoted as an "effector" substance (1). The same kind of question may be raised with regard to increased protein and RNA localization denoted as evidence of "synthesis" (1). However, such speculations must be classed as semantic hair-splitting until additional studies are carried out. The basic observations of Abeles and Holm are borne out by these experiments.

In the course of studying RNA and protein distribution, a marked increase in numbers of nuclei in the abscission zone was apparent. Nuclei in ethylene-treated material were conspicuous by their large size, and after treatment with ethylene, binucleate cells were commonly observed. Cell counts in the cortex, starchy sheath and pith in the abscission zone indicated an increased frequency of binucleate cells following 4 or 8 hour ethylene treatment (table I). Such an increase in the binucleate condition concomitant with ethylene treatment could arise in one of two ways: either as a result of mitotic divisions during that period which were not followed by cytokinesis; or as a result of the dissolution of existing middle lamellae and walls between adjacent cells during ethylene treatment. The latter is a particularly intriguing idea, since it is reminiscent of the manner in which bi- and polynucleate cells arise in the senescing tapetal layer in anthers, and the general observation of a binucleate condition is particularly interesting in view of a previously reported interference by ethylene of cell division (11).

Anatomical Studies. These experiments with ethylene thus demonstrated some interesting effects which probably are involved in the process of abscission. However, in order to properly evaluate the results of the physiological observations, the normal sequence of cellular events in the abscission of leaves on the intact plant must be established. These events must then be compared with cellular changes which occur in experimental material, and which are involved in the changing patterns of RNA and protein distribution. This problem was approached through a series of elapsed time studies of anatomical changes at the abscission zone in the intact bean plant from the time at which the primary leaf reached its maximum size (approx. 14 days after planting) until it abscised (30–34 days after planting); and in the explant, from the time of excision (14 days) to the time of abscission. In ethylene-treated explants abscission occurred from 28 to 32 hours after cutting; air-exposed explants abscised after approximately 50 hours.

In the region of separation in freshly cut explants, cells exhibited little morphological distinction. Cell walls were intact, cells were uniformly turgid, nuclei were normal in size, and no cell divisions

Table I. *Total Numbers and Distribution of Binucleate Cells in the Region of the Abscission Zone of* Phaseolus vulgaris[1]

Treatment	Tissue		
	Cortex	Starch sheath	Pith
Control	26	20	14
24 hr	29	22	18
24 + 4 hr C_2H_4	65	45	23
24 + 8 hr C_2H_4	60	44	31

[1] Comparable 1 mm region counted for each treatment. (100 ×-sections at 10 μ for each.)

Key to labeling of figures:[1]
AB—axillary bud
AM—amyloplast
AMB—amphicribral bundle
AR—abscission region
AW—anticlinal wall
C,CW—cell wall
COT—cotyledon
CP—chloroplast
CR—crystal
CX—cortex
CY—cytoplasm
D—distal
EP—epidermis
ER—endoplasmic reticulum
GB—Golgi body
I—invagination (adaxial)
LT—leaf trace
M—mitochondrion
MI—microbody
MW—mother cell wall
N—nucleus
NC—nucleolus
P—plasmodesmata
PE—petiole
PI—plasmalemma invagination
PL—primary leaf
PR—proximal
PP—parenchymatous pith
PS—pectic substance
PU—pulvinus
PW—periclinal wall
S—separation
SP—sclerified pith
ST—starch
T—telophase; also tannin
TP—tonoplast
TY—tylose
V—vacuole
VS—vessel element

[1] All original photomicrographs represent longitudinal sections of bean plants.

were evident. Six to 8 hours after explant excision, cell divisions commenced in the outer cortical layers in those cells at and immediately proximal to the future region of separation (fig 19). Divided nuclei remained within the walls of the mother cell. Mitosis was followed by cytokinesis so that nuclei were separated from each other by tenuous, newly formed transverse walls (fig 20). Cell divisions continued through the cortex during the 24 hour aging period; the number of dividing nuclei reached a maximum 12 to 18 hours after excision.

Concomitant with the initiation of cortical cell divisions (6-8 hr after excision), walls in a single layer of cells in the pith and walls of a few xylem vessels adjacent to the pith cells were disrupted (fig 21). Cell division did not precede breakage of pith cell walls or of xylem elements; divisions in pith cells commenced 16 hours after excision and involved only those cells lying immediately proximal to the disrupted cells.

When 24 hour aged explants were treated with ethylene for 4 or for 8 hours, cell division ceased and a disruption of cortical cells occurred. This was brought about by the dissolution of the newly formed transverse walls within the mother cells, which resulted in the appearance of large numbers of binucleate cells in the abscission region (fig 22). Cell separation through these recently divided cells in the cortex then occurred by disruption of the mother cell walls (fig 23).

In air-exposed explants, divisions in cells at and immediately proximal to the region of separation continued in the cortex for approximately 40 hours. Nuclear divisions were followed by wall formation, as described above, so that recently divided nuclei were separated by transverse walls within the mother cell. Continued divisions resulted in the formation of tiers of cells within the mother cell walls (fig 24). Separation was evident 40 to 50 hours after excision, and appeared to involve the dissolution of the newly formed transverse walls, particularly those of the distal part of a tier. Large numbers of binucleate cells appeared, and the breaking of the mother cell walls ensued. Abscission was virtually complete after approximately 50 hours.

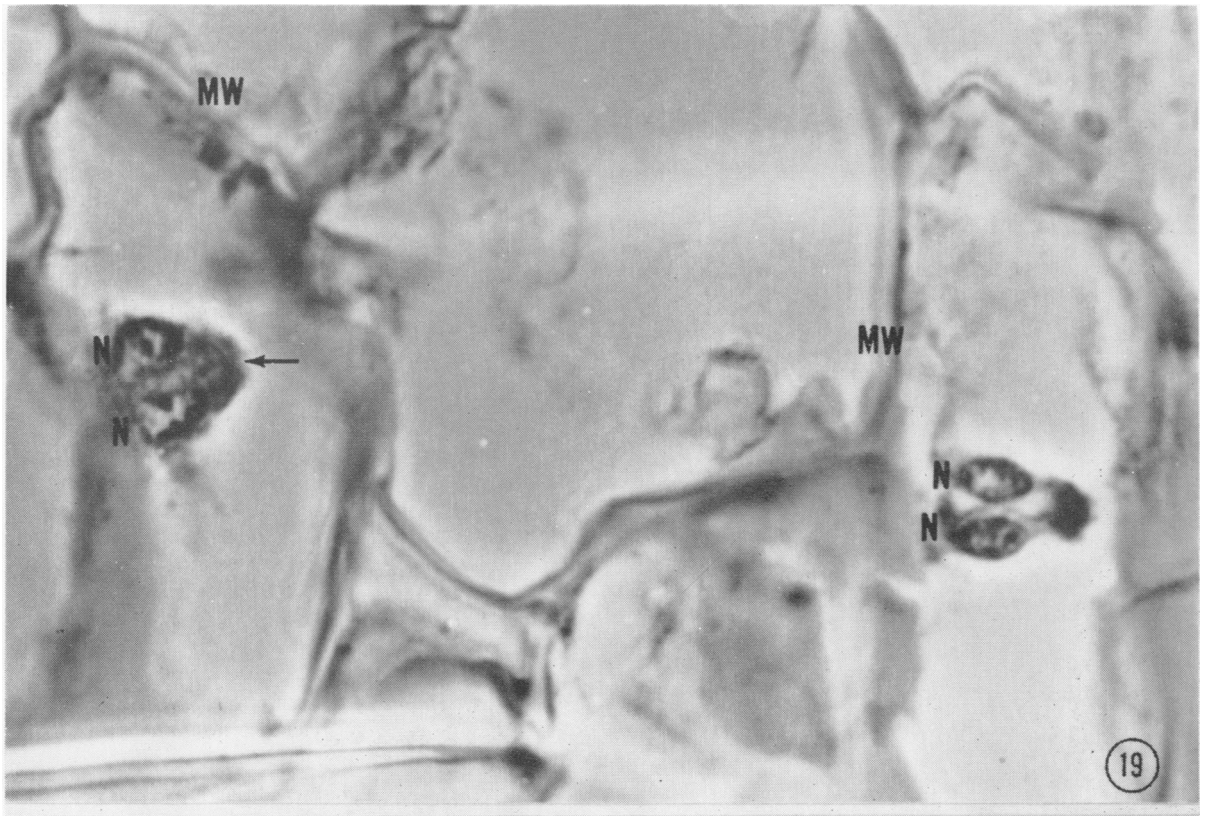
In the intact plant, anatomical changes which were observed were similar to those in air-exposed explants. In cortical cells at and immediately proximal to the region of separation, cell divisions commenced after about 19 days. Tiers of cells within the mother cell walls were apparent after 24 to 28 days. Breakdown of newly formed transverse walls, the appearance of the binucleate condition, and separation by breaking of the mother cell wall followed. Abscission occurred 30 to 34 days after planting.

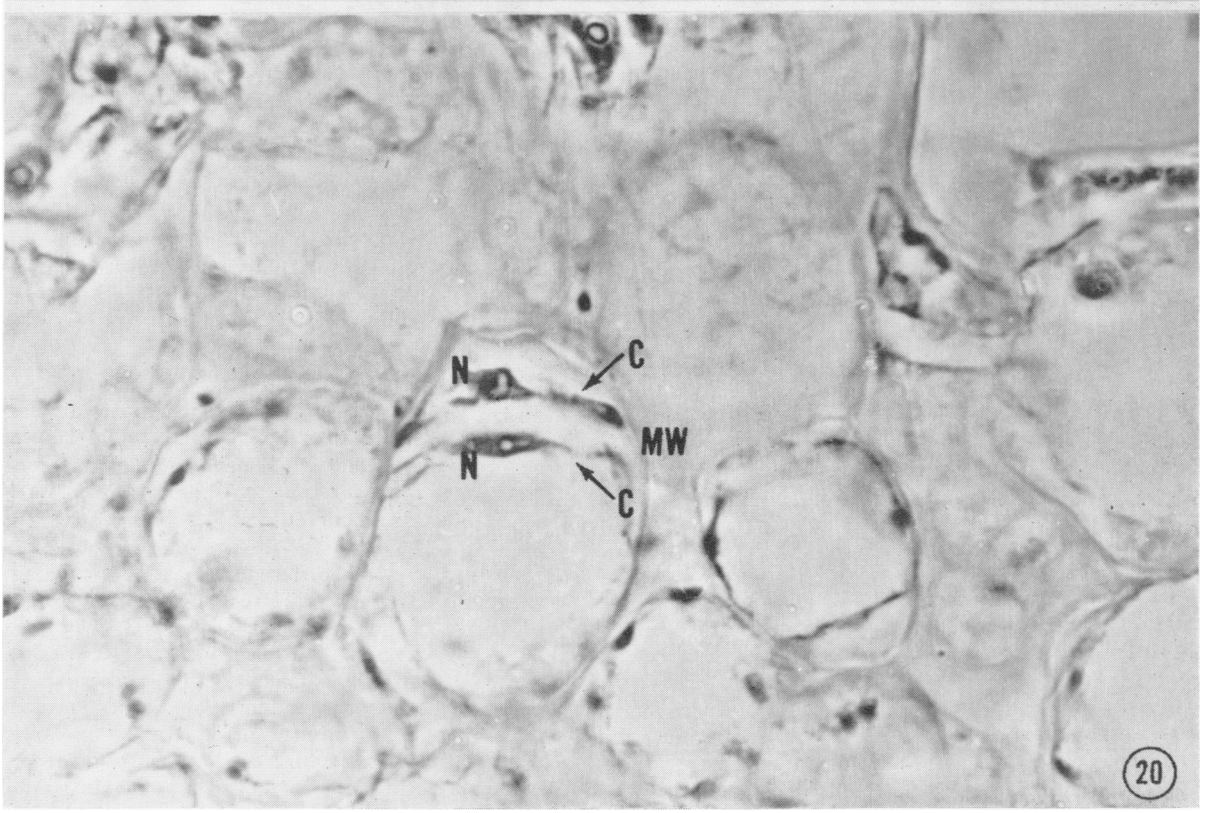
From these studies it appears that ethylene treatment *interrupts* a sequence of anatomical changes in the explant and that it *accelerates* a normal series of changes which ultimately result in cell separation. However, a causal relationship between cell division and ethylene effects on abscission is not necessarily implied here. One may well exist, but on the basis of these studies it has not been effectively demonstrated; in fact, there is some evidence that ethylene can stimulate abscission without cell divisions in tissues which normally do show divisions associated with abscission (11, 16, 23). The cell changes in the abscission region of *Phaseolus* do, however, appear to be related to separation, rather than to protection (16, 43); at the time of abscission recently divided cells were meristematic and unsuberized, and there was no evidence of periderm formation.

The actively dividing cells in the abscission region were those in which increased localization of nucleolar RNA and of nuclear and cytoplasmic proteins was noted. It is known that ribosomal RNA is synthesized in the nucleoli of actively dividing cell populations (5). The experiments reported here have demonstrated an increase in nucleolar RNA occurrence in those cells at or immediately proximal to the abscission zone. This correlation between site of increased RNA production and position of the line of separation lends support to the contention of Abeles and Holm (1) that RNA synthesis (?) is necessary for abscission, but of course it leaves entirely unexplained the causal mechanism between ethylene action and the abscission process.

On the basis of what is already known and what has been observed in these studies of the effects of ethylene, I would suggest that in freshly cut explants of *Phaseolus* endogenous levels of ethylene are high. Thus, applied ethylene is ineffectual in stimulating abscission and cell division is inhibited. After 6 to 8 hours of aging, endogenous ethylene levels at the abscission region might decrease to some critical level, at which time cell divisions commence. Subsequently, applied ethylene results in the cessation of cell division, and, in the cortical cells of *Phaseolus*, is followed by a series of cell wall changes ultimately leading to cortical cell separation.

I would also suggest that cell separation in the pith may not be causally involved in petiolar abscission, since it occurs soon after excision and before the majority of cellular changes occur in other tissues. In this regard, I am intrigued by Carl Leopold's discussion of differences in growth of tissues on each side of the abscission zone, and his suggestion that such differences appear to bring about the development of stress forces across the cell walls of the abscission zone (25). Ethylene-treated bean explants show a marked increase in width of the tissues proximal to the separation zone, and the swelling is not matched by tissues on the distal side. That difference in *Phaseolus* explants may account for the rupture of the proximal parenchymatous pith cells adjacent to distal sclerified pith cells. On the other hand, I regard cortical cell separation as an integral part of the abscission





FIGS. 19-20. Anatomical changes in the region of separation of the bean explant during abscission. Figure 19) Mitotic divisions (telophase) of nuclei in the outer cortical cells 6 to 8 hours after excision of the explant. Arrow indicates cell plate. ×3200. Figure 20) Recently divided nuclei in cortical cells of the explant, retained within the walls of the mother cell and separated by newly formed transverse walls. ×2000.

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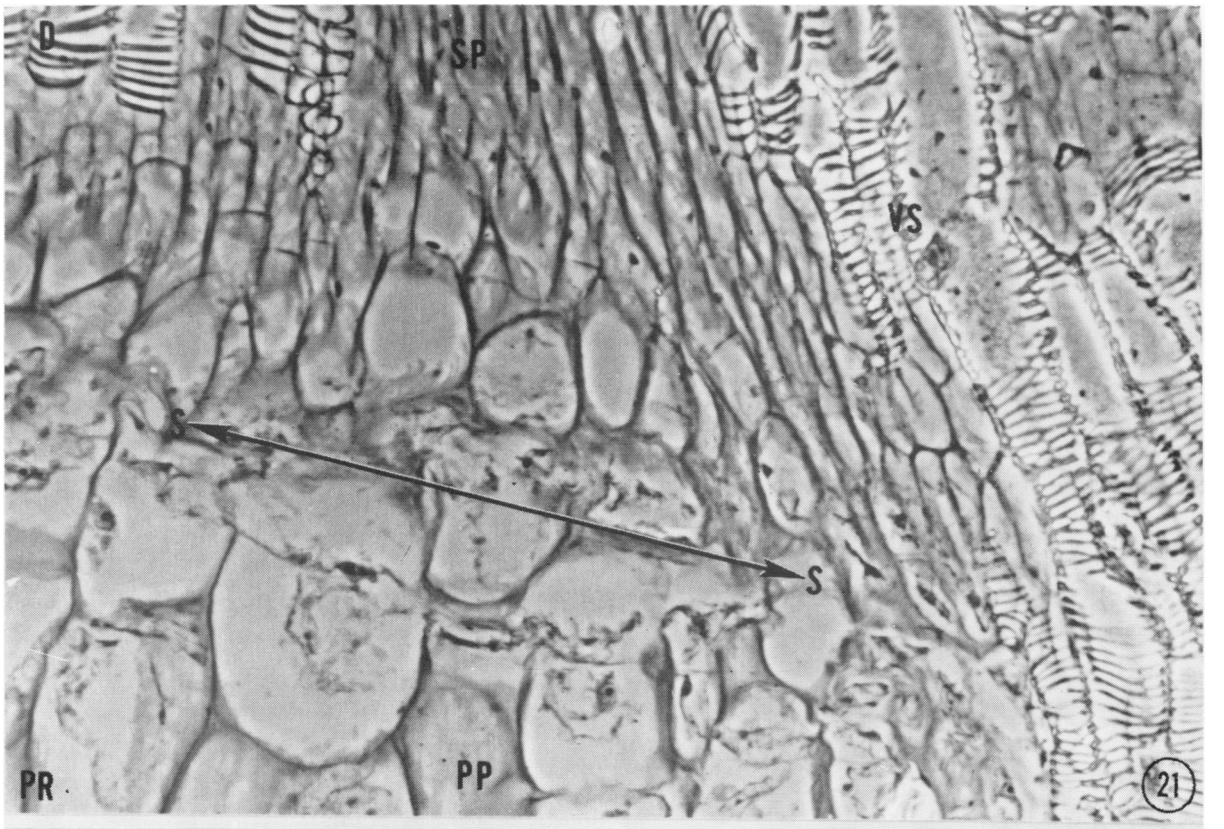

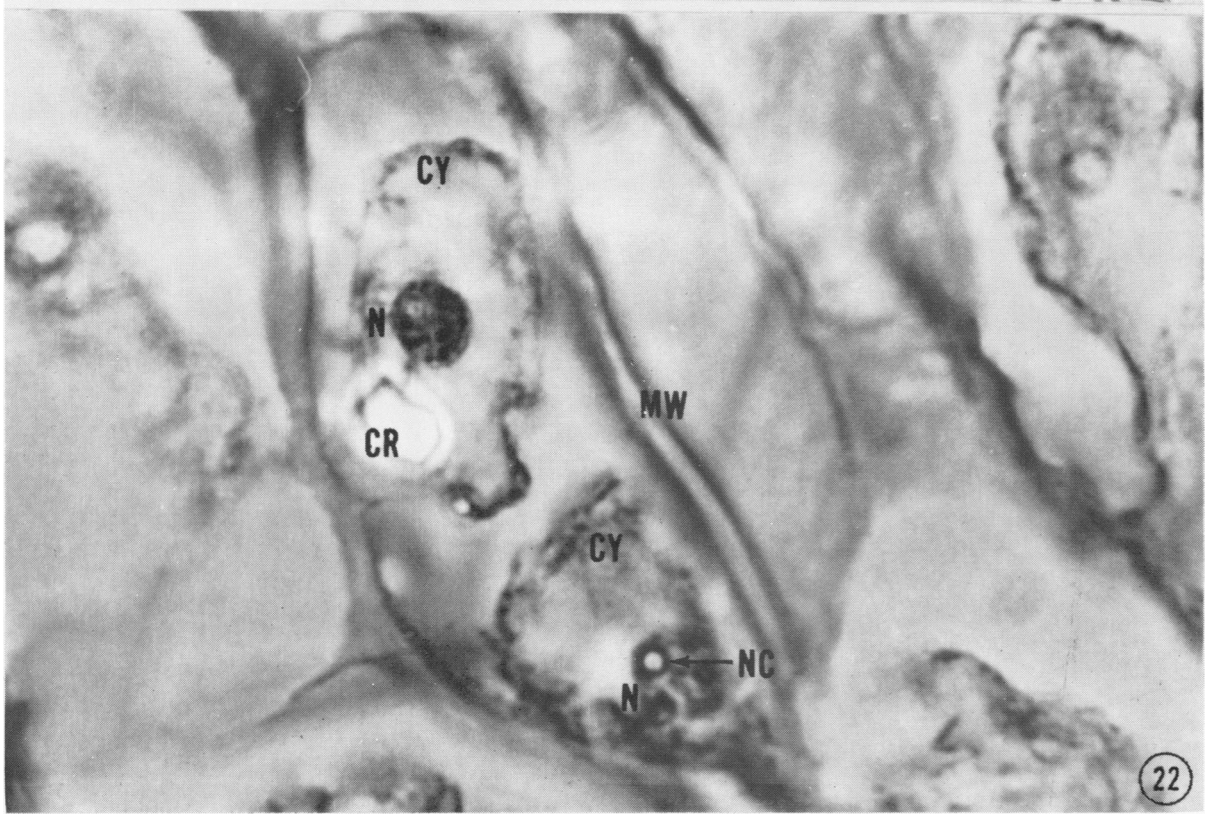

FIGS. 21-22. Anatomical changes in the region of separation of the bean explant during abscission. Figure 21) Disruption through a single layer of pith cells 6 to 8 hours after excision. ×1300. Figure 22) The binucleate condition of a cortical cell, after 8 hours of ethylene treatment. Note that each nucleus and its surrounding cytoplasm remains separate after the disappearance of the intervening cell wall. ×3200.

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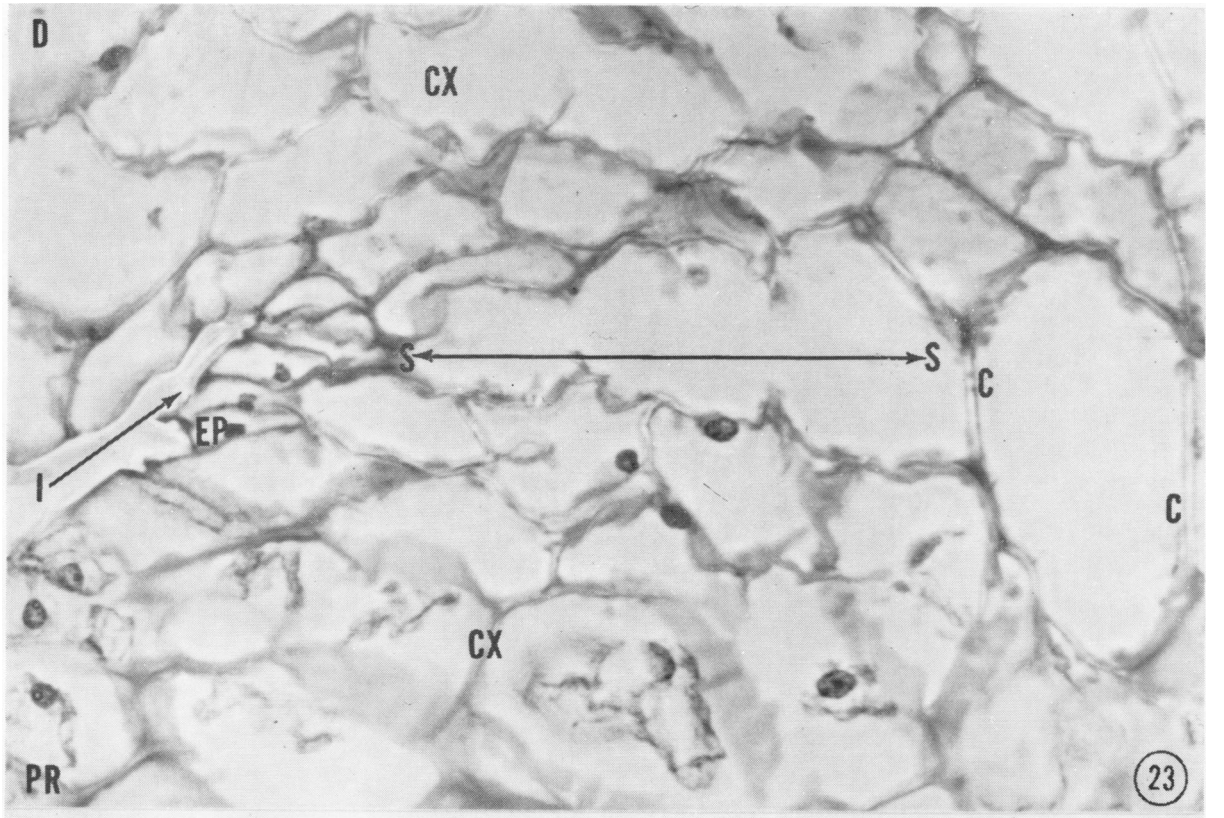
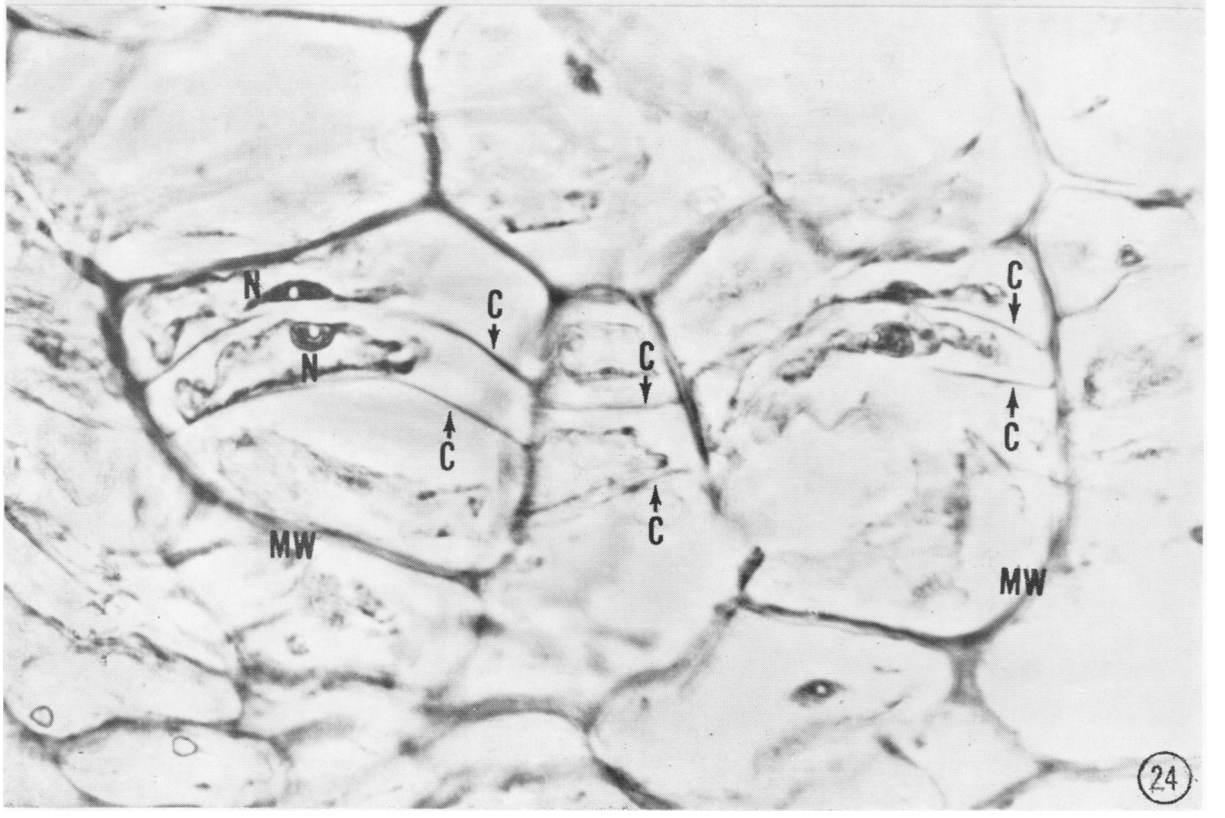
FIGS. 23-24. Anatomical changes in the region of separation of the bean explant during abscission. Figure 23) Cortical cell separation, after 8 hours of treatment with ethylene, through recently divided cells by breakdown of cell walls. Note the invaginated region, which occurs on the adaxial surface at the juncture of the pulvinus and the petiole. Note also that adaxial epidermal cells are still intact after separation has occurred internally. ×1300. Figure 24) Cortical cells of air-exposed explants 40 to 50 hours after excision. Nuclear division has been followed by new cell wall formation (arrows), and nuclei are retained within the walls of the mother cell. ×2600.





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FIGS. 25-28. Electron micrograph of cells in the abscission region at the cotyledonary node of cotton explants. Tissue was stained with alkaline hydroxylamine-$FeCl_3$. Figure 25) Tissue of freshly cut explant, showing the localization of pectic substances mainly in the middle lamella. ×11,200. Figure 26) Tissue from gibberellic acid (GA_3)-treated explant after 42 hours. The cell in the separation layer shows a decrease in staining of pectic substances in the region where breakage will occur. ×3900. Figure 27) Tissue from gibbereilic acid (GA_3)-treated explant after 42 hours. Note the loss of integrity of the cell wall through the loss of pectic (and other) substances, and the break in the cell wall. ×5700. Figure 28) Tissue from IAA-treated explant after 120 hours. Cell in the proximal layer with the cell wall and several cell inclusions still intact. ×7000. (Figs 25-28 from C. H. Bornman, 1965.)


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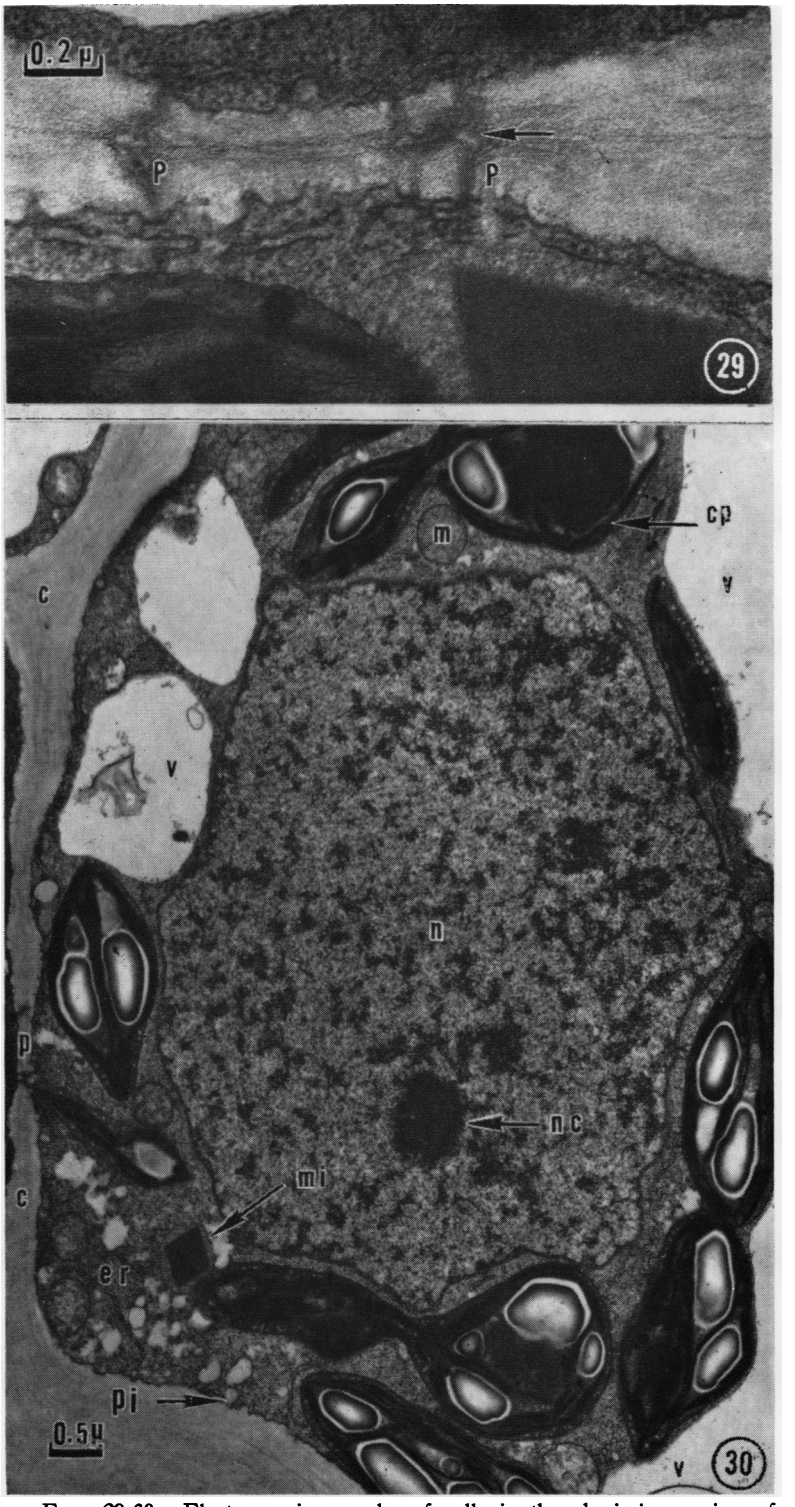

FIGS. 29-30. Electron micrographs of cells in the abscission region of *Nicotiana*. Figure 29) A portion of the cell wall showing plasmodesmata branching (arrow) in the region of the middle lamella. ×50,400. Figure 30) A section through a cortical cell in the abscission zone, showing various cell inclusions, including a microbody with a crystalloid core. ×8600. (Figs 29-30 from T. E. Jensen and J. G. Valdovinos, 1967.)

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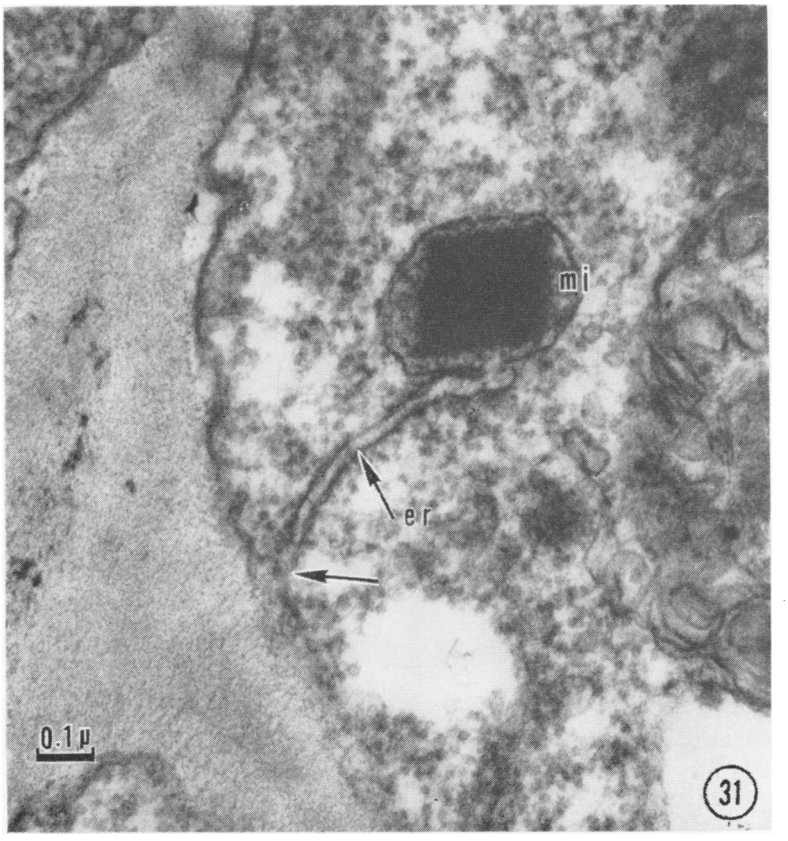

FIG. 31. Electron micrograph of a cell in the abscission region of *Lycopersicon*, showing an apparent connection of a microbody with a crystalloid core to a segment of endoplasmic reticulum which appears to be continuous with the plasma membrane (arrow). ×73,920. (From T. E. Jensen and J. G. Valdovinos, 1967.)

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process, and presume it to be influenced by ethylene application.

Abscission studies of *Phaseolus* petioles consistently show a markedly asymmetrical time relationship among the treatments. The ethylene- and nonethylene-treated explants are relatively close in elapsed time to abscission (32 *vs.* 50 hr), whereas the time to abscission in the intact plant is an order of magnitude greater (34 days). I would like to suggest on this basis that cutting of the explant triggers off a sequence of changes leading to abscission which may far outweigh the effects of experimental ethylene application. It would be of great interest to establish the changes in endogenous ethylene levels during abscission in the intact plant; Stanley Burg has pointed up in his symposium the pertinence of such observations. Correlation of these intrinsic changes in ethylene levels with those revealed during experimental treatments may well provide the key to elucidating the role of ethylene, as well as that of other substances, in the dynamics of the abscission process.

There are a number of similarities between the sequence of cellular changes which we have noted during abscission in *Phaseolus* explants and those which have been reported to occur in *Gossypium* explants (6). At the cotyledonary node of cotton, prior to abscission cell divisions give rise to two or more daughter cells, and cell wall rupture takes place along or through the newly formed cell walls.

Cell Wall Studies. It is well known that the cell walls of the separation region are chemically altered during leaf abscission in such a way that cells separate from each other or are easily broken (14). Sampson (35), Facey (15), Brown and Addicott (11) and others have noted changes in pectic substances preceding and during abscission, and Bornman (6, 7) has reported a histochemical study of cell wall changes in cotton at the electron microscope level. In freshly cut explants, staining with alkaline hydroxylamine-$FeCl_3$ indicated a localization of pectic substances mainly in the middle lamella (fig 25). After 72 hours, cells in the separation layer showed a decrease in staining of pectic substances in the wall prior to breakage; at that time there were also fewer mitochondria and Golgi bodies. In explants treated with gibberellic acid (GA_3), a decrease in staining was apparent after 42 hours, indicating an almost complete disappearance of pectic substances by that time. This was rapidly followed by cell wall separation (figs 26, 27). In explants treated with indoleacetic acid, there was some decrease in pectic substances but the integrity of the cell wall was maintained for a much longer time (120 hr) and most of the cell inclusions (starch, mitochondria and Golgi bodies) were still intact at that time (fig 28). Sequential extraction procedures indicated that in addition to pectic substances, a certain proportion of hemicelluloses was removed from the cells of the separation layer during abscission. Gibberellic acid greatly enhanced this process. Bornman summarized these observations by suggesting that wall breakdown occurred in 2 stages: an initial phase of cell separation consisting of the removal of pectic substances, followed by a breakdown of the cell wall polysaccharides. This resulted in weakening of the cell walls, and rupture followed. At the time of separation in *Gossypium* as in *Phaseolus,* no well-defined protective layer was present.

Recently a further refinement of fine structural cell wall studies has been reported by Jensen and Valdovinos (19). In a study of the zone of separation in flower pedicels of *Nicotiana,* they observed plasmodesmata with branches into the region of the middle lamella of the cell wall (fig 29). They speculated that dissolution of the middle lamella between cells in the abscission zone might be initiated at sites in the wall where plasmodesmata occurred. This would appear to be a promising direction for future research in *Nicotiana,* in view of the elaborate studies by Livingston (27) on the distribution of plasmodesmata in various cells of the tobacco plant.

Further studies by Jensen and Valdovinos (19) were concerned with observations of certain cellular inclusions and the separation processes of abscission (fig 30). Of particular interest were numerous microbodies with crystalloid cores which were occasionally observed in continuity with the cell wall (fig 31). The authors speculated on the possibility of a special relationship between the microbodies and cell separation processes characteristic of abscission.

Thornton and Thimann (41) noted similar crystalline bodies in *Avena* coleoptiles and suggested an involvement in phototropic responses. Cronshaw (12) showed crystalloids in *Eucalyptus* and *Acer,* and remarked that they were comparable to the microbodies with crystalline cores in rat liver cells, which de Duve (13) observed to contain hydrolytic enzymes. In view of the continuity of some of the microbodies in *Nicotiana* with the cell wall, it is interesting to speculate on their possible function in the abscission process.

This concludes the discussion of some contemporary anatomical aspects of leaf abscission. In addition to imparting specific details, I hope that it has provided some insight into the framework within which the plant anatomist may effectively pursue the problems of abscission.

Acknowledgment

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