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THE STYLAR TRANSMITTING TISSUE

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SUMMARY

The differentiation of the transmitting tissue of the style of *Petunia hybrida* was investigated with the electron microscope. The cells of this tissue are surrounded by an intercellular substance. The same holds true for a number of styles of other investigated species. This intercellular substance may not be compared with a middle lamella. The earlier suggestion, that the transmitting tissue of solid styles is collenchymatic in nature, is rejected.

1. INTRODUCTION

Solid styles with transmitting tissue are characteristic for most dicotyledons (HANF 1936). In this type of styles pollen tubes grow intercellularly, i.e. between the cells of the transmitting tissue or through the wall of these cells towards the ovules (JENSEN & FISCHER 1969). According to light microscope studies of the styles of *Lythrum salicaria* (SCHOCH-BODMER & HUBER 1947) the cells of the transmitting tissue have collenchymatic wall thickenings containing pectin, hemicellulose and cellulose. Pollen tubes are assumed to dissolve these collenchymatic thickenings while growing through the middle lamella (SCHOCH-BODMER & HUBER 1947). EM-studies of pollinated and unpollinated styles of *Petunia hybrida* (VAN DER PLUIJM & LINSKENS 1966, KROH & VAN BAKEL 1973) and *Diplotaxis tenuifolia* (KROH & MUNTING 1967) showed that the cells of the transmitting tissue are surrounded by a substance probably pectinic in nature, which is dissolved by the growing pollen tubes. Therefore the collenchymatic nature is not generally found in transmitting tissue of solid styles.

The present work deals with the differentiation of the transmitting tissue in the styles of *Petunia hybrida*. Our attention was especially focused on the intercellular substance. Moreover the transmitting tissue of several other plant species is compared with that of *Petunia*.

2. MATERIAL AND METHODS

Styles of *Petunia hybrida* were harvested at several stages of flower development by cutting just above the ovary and below the stigma.

The smallest styles were about 0.5 mm, the full-grown ones about 32 mm in length. They were cut into pieces of about 1 mm, fixed with glutaraldehyde, postfixed with $KMnO_4$, and embedded in Epon. Only the middle parts of the pieces were used for sectioning since preliminary work revealed considerable deformation of the transmitting tissue by cutting.

The other plants from which styles were investigated include: *Capsella bursa-pastoris*, *Lythrum salicaria*, *Lythrum virgatum*, *Vitis vinifera*, and *Tradescantia virginiana*. From these plants one stage of development was chosen, the stage just before anthesis.

In order to detect the location of the cellulose component in the transmitting tissue, styles were treated with a mixture of hydrogen peroxide and acetic acid (1:1) for half an hour, embedded in butyl metacrylate and sectioned. The butyl metacrylate was removed from the sections with amylacetate and the remaining cell wall component shadowed with platinum.

To investigate the pectin component in the intercellular substance of the transmitting tissue, style pieces were treated for half an hour with a 3% pectinase solution in 0.01 N acetate buffer, pH 4.4. For controls untreated styles and styles treated with buffer and with distilled water were used.

After this treatment the styles were fixed and embedded in Epon as described above. The investigation was performed with a Philips EM 300 electron microscope.

3. RESULTS

Beginning with young styles (0.5–1 mm) it was found that cell division generally does not occur; from this stage onwards only cell elongation is found.

Fig. 1 shows a longitudinal section from the transmitting tissue of a young *Petunia* style. The cells are lined up in the longitudinal direction. A relatively large nucleus and organelles are found as in young plant cells. The transverse cell walls are very thin and traversed by numerous plasmodesmata. The longitudinal cell walls appear to be thicker, but the primary wall is thin and the intercellular substance can be distinguished in some areas. The longitudinal cell walls possess fewer plasmodesmata than do the transverse walls.

Fig. 2 shows a transverse section of the transmitting tissue of a *Petunia* style of about the same length (0.5–1 mm). Large parts of the lateral cell walls are still connected with each other, but at the corners intercellular spaces originate. In contrast to the usual situation in growing tissue, these intercellular spaces are not empty but filled with an intercellular substance with higher electron density than the primary walls. This substance apparently has been secreted by the cells of the transmitting tissue. The lateral cell walls still contain plasmodesmata on surfaces where they remain in contact. Starting from this stage the amount of intercellular substance gradually increases, but the highest increase is observed just before anthesis. The direct contact of the cells in transverse direction disappears and at the same time the plasmodesmata disappear. A transverse section of a style about 30 mm in length is seen in *fig. 3*. The cells have increased about 20 times in length, but the diameter has not increased. In the cells large vacuoles are seen to originate, but they never occupy the whole central part except at the cell ends.

The increase in intercellular substance, as measured from 3 or 4 photographs of transverse sections of transmitting tissue, is shown in *table 1*. The surface

Table 1. Increase of intercellular substance of the transmitting tissue from *Petunia* styles before and during anthesis. For each stage 3 or 4 photographs have been used for measuring.

Stage of style	surface intercellular substance / surface occupied by the cell				average
	1	2	3	4	
style 27–28 mm	1.03	1.10	1.11	–	1.08 ± 0.04
flower closed	1.29	1.20	1.22	1.17	1.22 ± 0.05
flower half open	1.38	1.40	1.45	–	1.41 ± 0.04
flower almost open	1.58	1.75	1.70	1.68	1.68 ± 0.07
flower open	1.99	2.07	1.90	1.92	1.97 ± 0.08

occupied by the cells did not decrease during this development. From measurements we can conclude that there are no remarkable changes in the number of organelles which may be involved in synthesis of intercellular substances.

Fig. 4 gives a detail of a stage shown in *fig. 3* (30 mm style). The cells contain the usual population of cell organelles. Occasionally plasmodesmata are found in the lateral walls. The cell wall shows an inner part which is more electron dense than the outer part. The intercellular substance has a higher electron density than the outer part of the wall. After treatment with pectinase the transmitting tissue (from the same stage as *fig. 4*) shows a dissolution of the intercellular substance *(fig. 5)*. Now the primary cell wall is clearly seen. The cells have apparently not been affected by the enzyme treatment. Styles treated with buffer or with water only, also revealed a slight dissolution of the intercellular substance.

Looking at the transmitting tissue of styles of other plants we may conclude that this is, roughly speaking, the same as that of *Petunia*. The transmitting tissue of *Capsella bursa-pastoris (fig. 6)* contains many plasmodesmata in the lateral walls, this in contradistinction with the *Petunia* styles. These plasmodesmata traverse the intercellular substance, where we often see them in transverse section. The cell walls are rather thick and not well stained. *Lythrum salicaria (fig. 7)* does not have such plasmodesmata. As in *Petunia*, the primary cell wall is again subdivided into two layers, the inner one being more electron dense. In contrast *Lythrum virgatum (fig. 8)* does not show plasmodesmata connecting the lateral walls or the two layers in the primary wall. The primary cell wall is rather thin and can be compared with that of *Petunia*. The transmitting tissue of *Vitis vinifera* styles *(fig. 9)* shows a very thick primary cell wall. It is not subdivided into two layers but on the outside it is covered by a more electron dense layer which may be the intercellular substance.

In order to investigate the cellulose component of the transmitting tissue, the styles were treated with hydrogen peroxide and acetic acid. By this treatment the cytoplasmic and the cell wall constituents except cellulose are dissolved. *Fig. 10* shows the cellulose skeleton of the transmitting tissue of a style of

Lythrum virgatum. It is evident that there is more space in between the cells of the transmitting tissue than in surrounding parenchymatic tissue. Since the intercellular substance is no longer present, the cells may clump together and show an uneven distribution. This photograph and *fig. 11* show clearly that cellulose is only present in the primary cell wall of the transmitting tissue and not in the intercellular space. For comparison (see discussion) two pictures from a style with an open style canal are placed side by side. *Figure 12* shows a transverse section of a young style of *Tradescantia virginiana*. The inner side of the canal is lined by a layer of cells comparable with the cells of the transmitting tissue of the solid styles. The cell surfaces directed towards the canal are covered with a cuticle that was artificialy detached from the cell walls. The rest of the stylar tissue is built up of parenchyma cells. In this young stage the canal is still empty, but when the flower opens a secretion product is found in the canal *(fig. 13)*. The question whether this secretion product is comparable and perhaps similar to the intercellular substance of the transmitting tissue will be discussed.

4. DISCUSSION

In this investigation our attention was focused on the cell walls of the transmitting tissue of styles of different plants. In textbooks of plant anatomy (for instance ESAU 1965) the transmitting tissue is called a collenchymatic tissue. This description originates from a light microscope investigation of stylar tissue by SCHOCH-BODMER & HUBER (1947). From the microscopic morphology of transmitting tissue of *Petunia* (VAN DER PLUIJM & LINSKENS 1966) and of *Diplotaxis* (KROH & MUNTING 1967) we know that in these plants collenchyma and transmitting tissues are not comparable, but it was not certain whether this holds true for the styles of other plant species. Among other plants, *Lythrum salicaria* was investigated because the conclusions of Schoch-Bodmer & Huber were based on the stylar tissue of this plant. However, in this species, as in the others, the transmitting tissue does not show a collenchymatic nature. From the species which have been investigated it must be concluded that the transmitting tissue cannot be compared with collenchymatic tissue. In the latter the primary wall thickenings, in the form of cellulose lamellae, alternate with pectin layers and are laid down in a centripetal pattern. In the transmitting tissue of styles there are no wall thickenings but only a secretion product outside the primary wall. The difference between collenchymatic tissue and transmitting tissue is clearly shown in *figure 14*. The transmitting tissue in the style of *Gossypium hirsutum* (JENSEN & FISCHER 1969) is yet a different type. The cell walls are very thick and there is a normal middle lamella. However, there is no reason to compare these cells with collenchyma. In *Gossypium* the pollen tubes do not grow intercellularly but through the cell wall.

The intercellular substance in the transmitting tissue has been called an extremely thick middle lamella (VAN DER PLUIJM & LINSKENS 1966). It seems unlikely that the limited material present in the original middle lamella could form

such a mass of intercellular substance, while the electron density remains the same. This material must be excreted from the cells of the transmitting tissue and, in this case, the intercellular substance may not be called middle lamella. From an ontogenetic point of view the term middle lamella is reserved for the first part of the cell wall originating from fusing Golgi vesicles at cell plate formation. The middle lamella is completed when the primary wall starts growing. Furthermore the middle lamella contains pectin, while there is strong evidence that the intercellular substance from the transmitting tissue differs from the pectin of the middle lamella by its uronic acid composition portions (KROH 1973). From our experiment with pectinase it may not be concluded that the intercellular substance contains pure pectin, since we did not use purified pectinase and since treatment with buffer only and with water only also has a dissolving effect, though less than pectinase treatment. Furthermore, there is evidence from chemical analysis that the composition of intercellular substance of *Petunia* styles is more complex than that of simple pectin (KROH 1973). It seems more logical to compare the intercellular substance with the mucilage in the canal of hollow styles (LABARCA, KROH & LOEWUS 1970). In terms of function, the difference between solid and hollow styles is not so great. Within one family, for instance the Amaryllidaceae (JOHRI 1966), species possessing one layer of transmitting tissue lining the inner side of the canal are found, but also species possessing two to six layers of transmitting tissue. Species with a partly solid style are also present in this family.

From this investigation and from earlier work (KROH & MUNTING 1967) it is clear that the intercellular substance does not contain cellulose. A cellulosic skeleton is only found in the primary cell walls of the transmitting tissue, the normal situation for parenchyma cells.

In summary we may conclude that:

1. The transmitting tissue of solid styles can not be compared with collenchymatic tissue.
2. The intercellular substance, if present, is not to be considered as a middle lamella, but rather as a secretion product of the transmitting tissue.
3. This secretion product may be compared with the mucilage that fills the canal of open styles.

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Key to labelling

tw	transverse walls	c	cuticle
lw	longitudinal wall	tt	cell of transmitting tissue
pl	plasmodesmata	pc	parenchymatic cell
is	intercellular substance	m	mucilage
pw	primary wall	cm	cellulose micrifibrils in primary wall
n	nucleus	cc	collenchyma cell
ics	intercellular space	ml	middle lamella
op	outer part of primary wall	g	Golgi apparatus
ip	inner part of primary wall	p	plastid
el	electron dense layer	er	endoplasmic reticulum
sc	style canal	v	vacuole

Legends

Fig. 1. Longitudinal section through the transmitting tissue of a *Petunia hybrida* style with a length of about 0.5–1 mm. Arrow points in the longitudinal direction.

Fig. 2. Transverse section of the same tissue as *fig. 1.*

Fig. 3. Transverse section from a *Petunia hybrida* style about 30 mm length.

Fig. 4. Higher magnification of the same section as *fig 3.*

Fig. 5. Transverse section of stylar tissue of *Petunia hybrida* after treatment with pectinase. Intercellular substance is dissolved.

Fig. 6. Transmitting tissue from a style of *Capsella bursa-pastoris.* Plasmodesmata traverse the intercellular substance.

Fig. 7. Transmitting tissue from a style of *Lythrum salicaria.*

Fig. 8. Transmitting tissue from a style of *Lythrum virgatum.*

Fig. 9. Transmitting tissue from a style of *Vitis vinifera.*

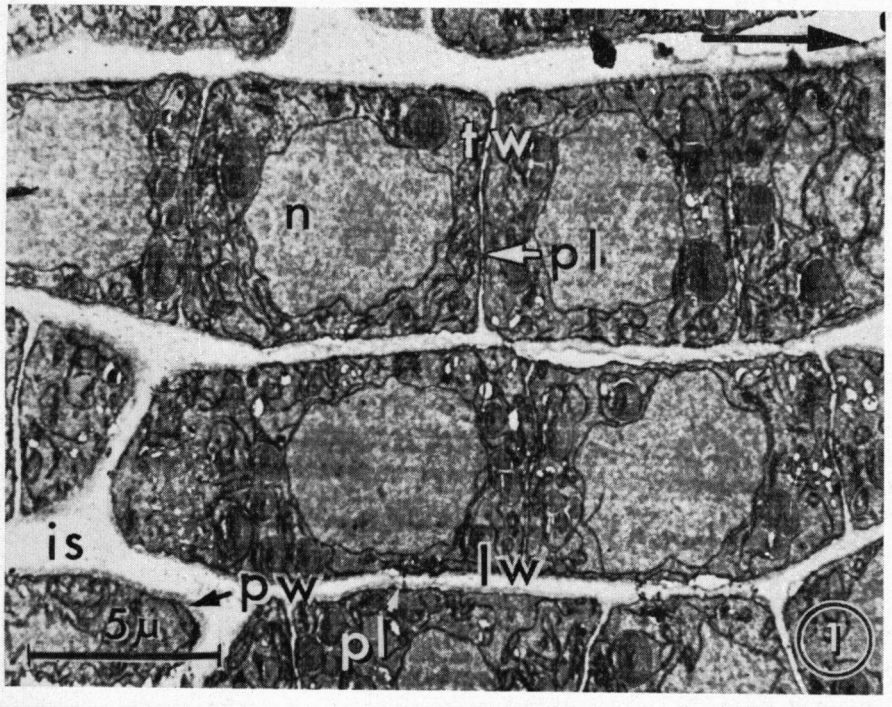
Fig. 10. Transverse section through stylar tissue of *Lythrum virgatum* after treatment with hydrogen peroxide and acetic acid. The encircled part is the transmitting tissue.

Fig. 11. Detail of the encircled part of *fig. 10* showing the cellulose microfibrils of the primary wall.

Fig. 12. Part of the hollow style of a closed flower of *Tradescantia virginiana.* The empty style canal is lined with one layer of cells comparable with the transmitting tissue of solid styles.

Fig. 13. Part of the style of *Tradescantia virginiana* but now from an open flower. The style canal is filled with mucilage.

Fig. 14. Schematic drawings of transmitting tissue, A and collenchyma tissue, B.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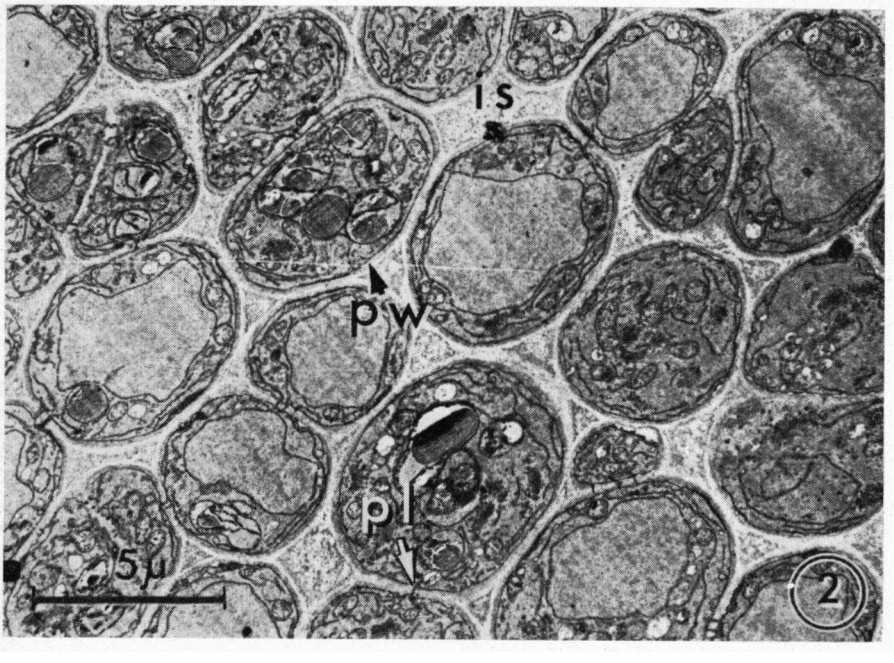
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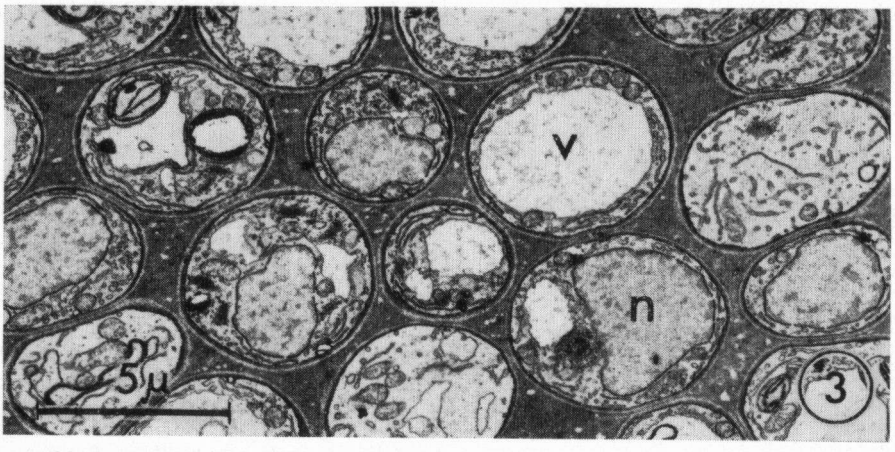
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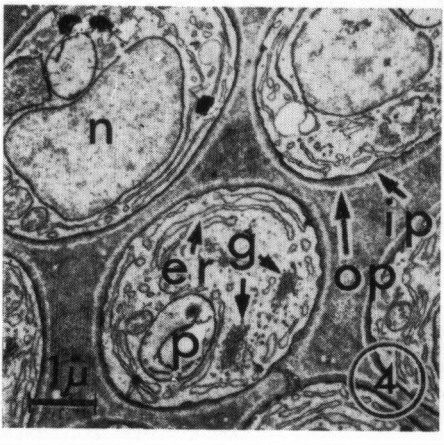
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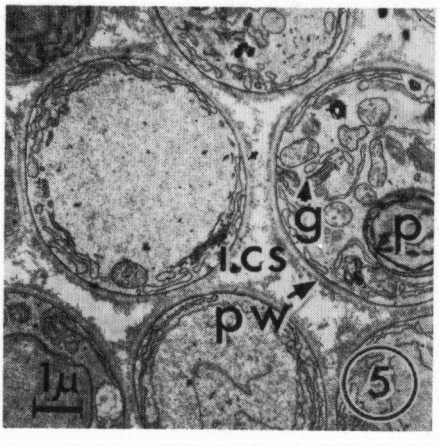
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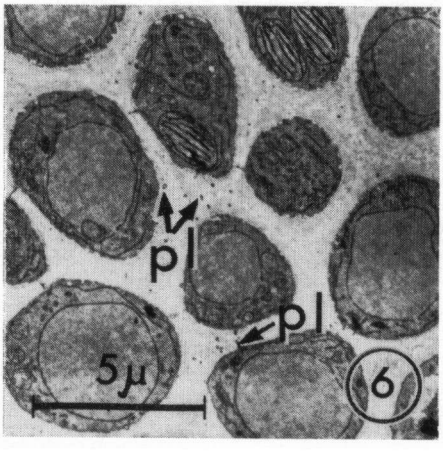
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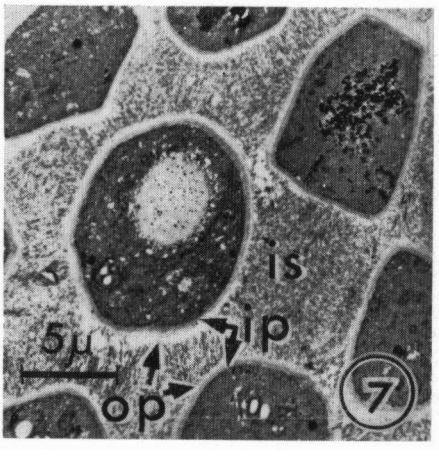
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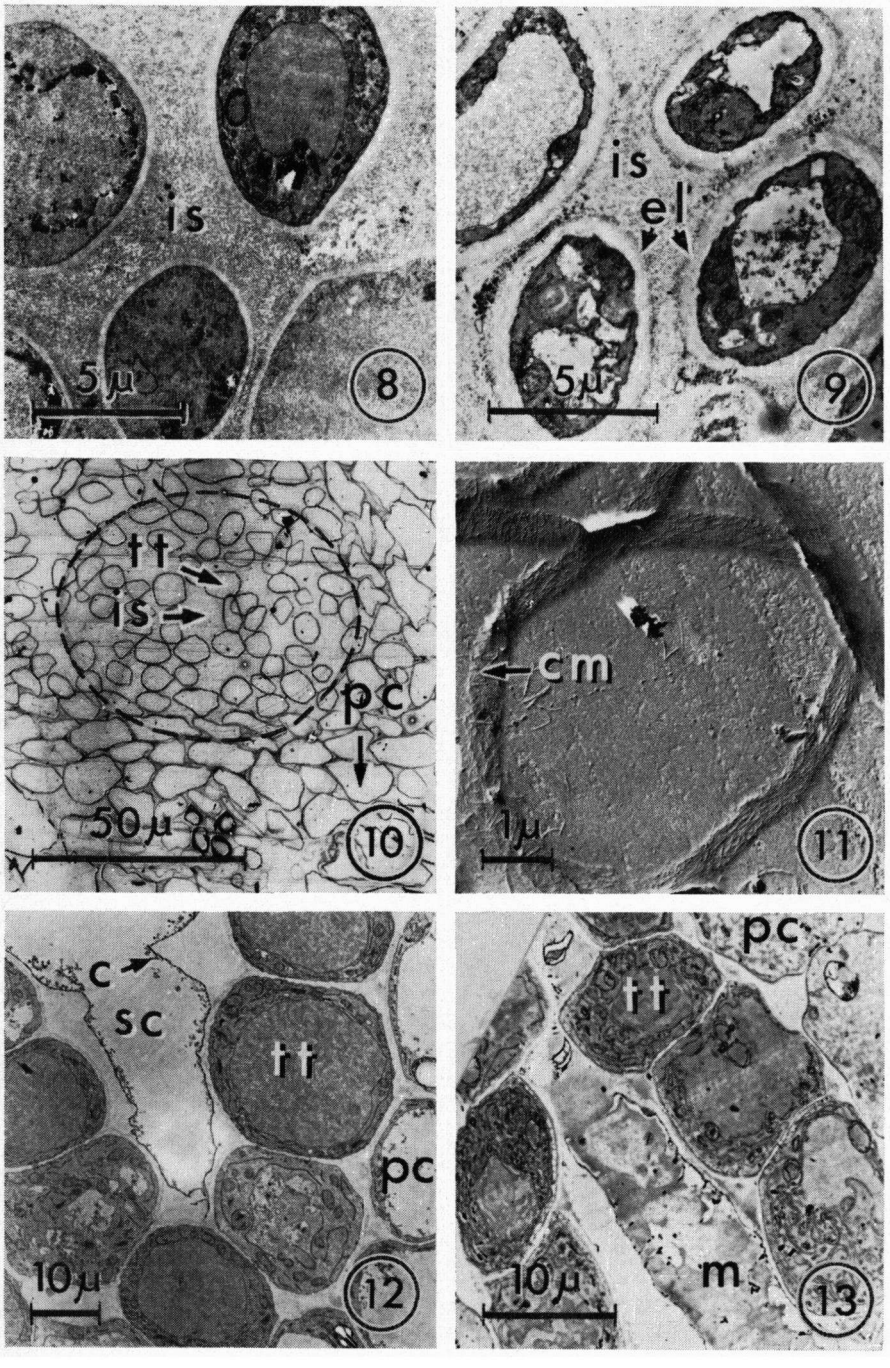
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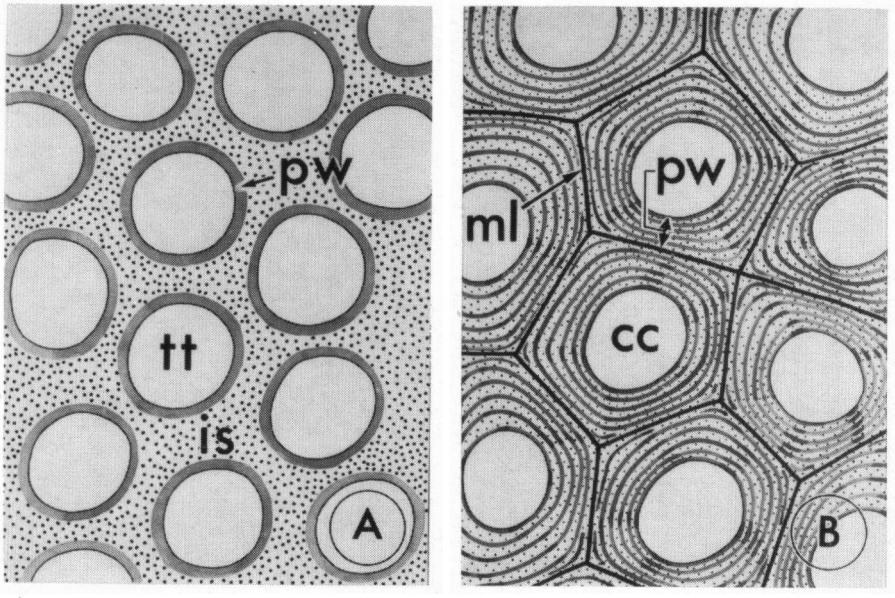

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