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Exine Development in *Borago* (Boraginaceae). 1. Microspore Tetrad Period

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ABSTRACT : In the tetrad period of *Borago officinalis* L. microspores, procolumellae (exine tuft-units) are 70-100 nm in diameter and have striated or coiled substructures. Tufts have a circular core zone that initially can be strongly contrasted and an outer (binder) zone that contrasts weakly. During increase in tuft height the early formed distal diameter of tufts becomes 150 to 200 nm while the new basal part is 70 to 100 nm. Thus for a time these procolumellae are cone-like. Along with this change in shape and height there is a reversal of contrast. For a time the core is only weakly contrasted while the binder zone can be strongly contrasted. It is the binder zone that is striated or coiled. Tectal formation involves elongation or elevation of units distally and reversal of contrast. The binder zone can be darkly contrasted. The procolumellae are now 0.4 to 0.5 μm in height from plasma membrane to the callose special cell wall. The distal portion of procolumellae interdigitate producing a polygonal pattern as seen in oblique sections. A tectum becomes prominent and a foot layer and endexine begin to be evident during the period of loss of the microspore mother cell callose envelope. The apertures become complex before the end of the callose period. As they are formed the plasma membrane is separated from the callose by only about 50 nm. At the margin of these young apertures the glycocalyx matrix tapers from the about 50 nm to the height of the interapertural procolumellae (about 150 nm). The callose of the special cell wall remains until the free microspore period.

KEY WORDS : Binder zone, *Borago*, Callose, Microspores, Glycocalyx units, Procolumellae, Tetrad.

INTRODUCTION

The paper by Ben Saad-Limam and Nabli (1984) is a major work on the structure of exine and aperture system of Borago officinalis. Their study includes splendid micrographs in stages after callose digestion. The *Borago* pollen grains are mostly subprolate. They are 33 to 40 μm in diameter and the usual number of apertures is 10. These colpi are narrow and 10 to 20 μm in length. One of the prize figures of the Ben Saad-Limam and Nabli (1984) paper is an SEM of transverse section through ten apertures. In addition to their own stimulating results Saad-Limam and Nabli gave a through review of the literature on *Borago* pollen beginning with the very early reports of Geoffroy (1711), Mohl (1834) and Aldridge (1842). Further light microscopical observations were reported by Erdtman (1952), Avetisian (1956), Stix (1964) and Bou (1968). The work of Stix (1964) with phase contrast microscopy

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showed the great complexity of exine architecture in *Borago* pollen as was also emphasized in Clarke's (1977) scanning microscopical observations.

Our aims in the study of *Borago officinalis* pollen were to consider the manner of establishment of the complex exine and aperture system, as well as formation of the decorated and seemingly unusual gemmae that cover the pollen tectum. Each of these subjects will be considered under appropriately entitled parts I covering exine initiation to end of tetrad period and II going from the early free microspore stage to mature pollen grains. Because Saad-Limam and Nabli's work covers stages after the tetrad period we plan to review it in our *Borago*-II manuscript.

MATERIALS AND METHODS

Buds of *Borago officinalis* L. were collected in the gardens of the Botany Department of Stockholm University. Entire anthers with attached filaments were immersed in one of three fixation mixtures (pH 7, 20C, 24 h) and immediately placed under vacuum (ca. 10-2 torr) for about 10 min or until bubbles were no longer released from the specimens and specimens had settled to bottom of vial. The fixation mixtures were then slowly rotated for the rest of the periods of fixation, dehydration and epoxy/acetone infiltration.

The three fixation mixtures were based upon Karnovsky's (1965) formulation of 2.5% glutaraldehyde and 7.5% paraformaldehyde in 0.06M phosphate buffer diluted 1:1 with distilled water, pH 7.2. They differed by addition of either 1% lanthanum nitrate (LN), 0.003% ruthenium red (RR), or 1% Alcian blue (AB). Addition of these cations was both in the initial Karnovosky mixture and in the osmium tetroxide secondary fixation. After the initial period the fixation mixtures were decanted and specimens transferred to 1% osmium tetroxide (dissolved in water, held at 20C, 1 hr) plus one of the above cations. After acetone dehydration the anthers were embedded in Spurr's (1969) hard resin.

To aid in selecting stages for TEM, unstained semithin (3-7 μm) sections were examined by phase contrast and differential interference contrast (Normarski) light microscopy. Unless otherwise noted in illustration descriptions, ultrathin (50-80 nm) sections were contrasted with a saturated solution of uranyl acetate in ethanol and 0.2% lead citrate (pH 12.2). Sections were examined with Zeiss EM-10A and Hitachi H-600 transmission microscopes.

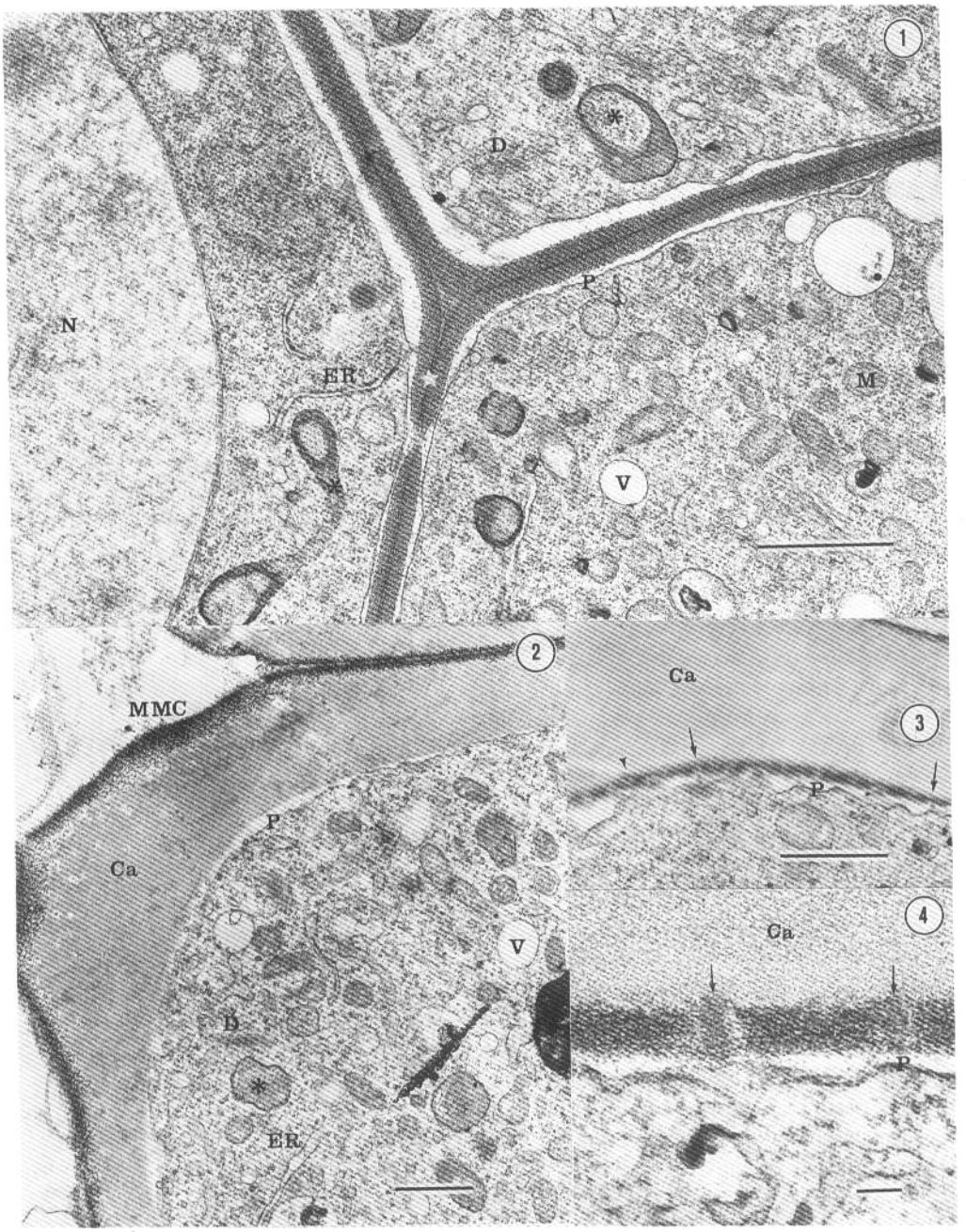
RESULTS

Early tetrad stage before exine initiation

Sporogenous cells are separated by cell walls with a distinct middle lamella (Fig. 1). After meiosis microspore tetrads are enclosed within callose (Fig. 2) of the microspore mother cells (MMC). There is an extensive MMC callosic envelope but no exine development (Fig. 2).

Early exine template

A highly contrasted glycocalyx matrix and plasma membrane glycocalyx units (procolumellae) are inserted against the callose (Figs. 3, 4). Procolumellae, 70 to 100 nm in

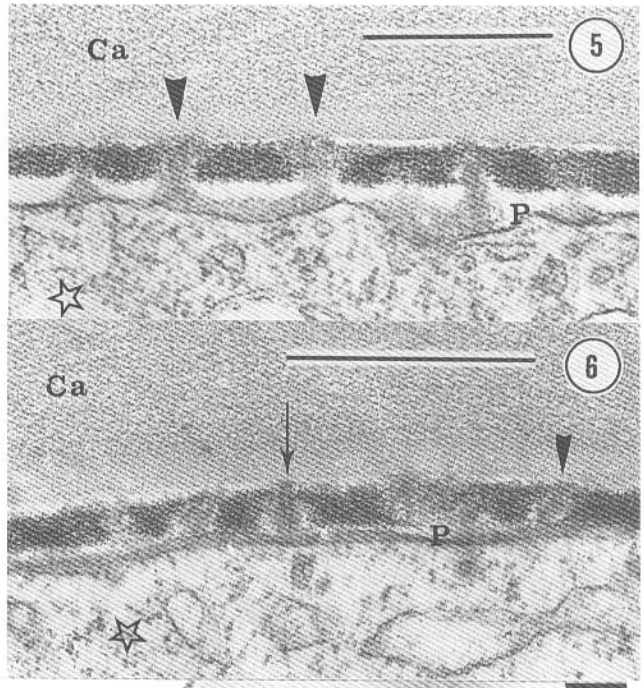


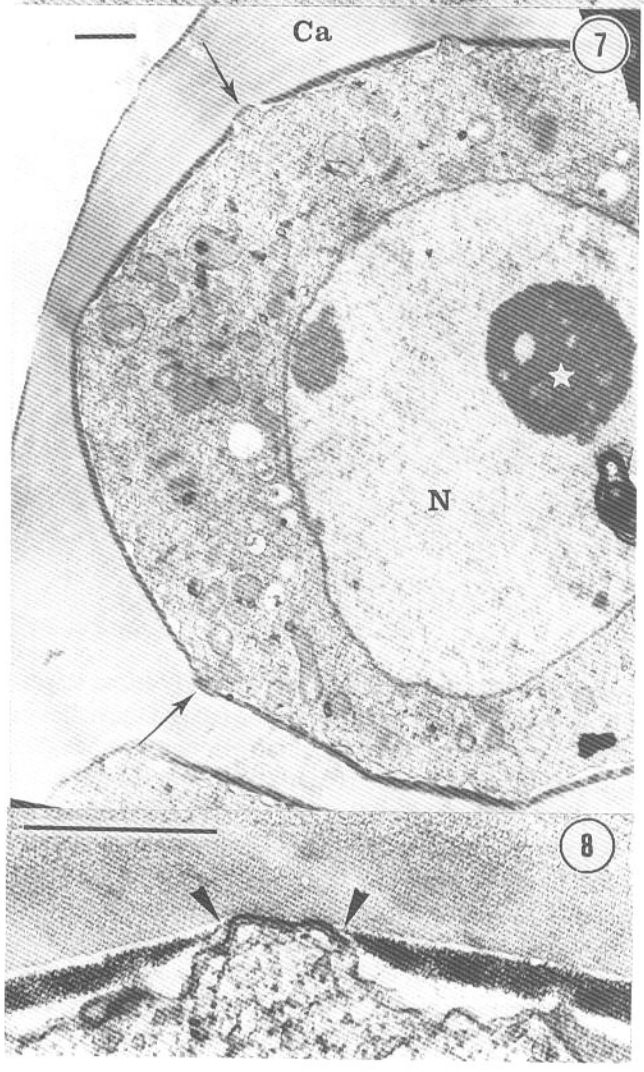
Figs. 1-4 Sporogenous cells (Fig. 1) and early tetrad stages (Figs. 2-4). Fig. 1. Sporogenous cells separated by cell walls with a distinct middle lamella (star). Organelles include amoeboid plastids (asterisks) many of which are highly convoluted, mitochondria (M), dictyosomes (D), endoplasmic reticulum (ER), and small vacuoles (V). The plasma membrane is marked (P) and the nucleus (N). Fixation: RR. Bar=1 μm (23,000 X). Fig. 2. Early tetrad stage. One of four very young microspores within an extensive microspore mother cell coating (MMC) and callosic envelope (Ca). The symbols for cytoplasmic organelles are the same as in Fig. 1. Fixation: LN. Bar=1 μm (13,000X). Fig. 3. Early tetrad stage. Darkly contrasted glycocalyx layer between the callose (Ca) and plasma membrane (P). Microspore with early development of glycocalyx units ("procolumellae") (arrows) from the plasma membrane. Some of the procolumellae are sectioned obliquely and can be seen to be circular or ellipsoidal in end views (arrowhead). Fixation: RR. Bar=1 μm (18,000X). Fig. 4. Early tetrad stage. Plasma membrane glycocalyx units (arrow) which are the procolumellae show cross striations. Callose (Ca). Fixation: RR. Bar=0.1 μm (75,000X).

diameter, are attached to the plasma membrane (Figs. 3-6). The first part of the units formed (presumably the future tectum) extends slightly out from the glycocalyx matrix (Figs. 3-6). With adequate magnification (Fig. 4) cross striations are apparent.

Formation of apertures

In equatorial section the surface of the early microspore is somewhat flattened between the aperture sites (Fig. 7). The plasma membrane of future apertures is separated from the callose by only ca. 50 nm thick-ness of dark stained glycocalyx (Figs. 8, 28).





Figs. 5-8. Details of the developing glycocalyx units (procolumellae). Fig. 5. Glycocalyx units extend from the plasma membrane (P) to the callose (Ca). Two procolumellae marked by arrowheads, sectioned medially, are ca. 100 nm in diameter and their height is ca. 200 nm. Microspore cytoplasm (star). Fixation: RR. Bar=0.5 μm (50,000X). Fig. 6. The plasma membrane (P) has a thick (50 nm) coating under the darkly contrasted glycocalyx at the stage shown in Fig. 5. The glycocalyx unit (procolumella) marked by an arrow runs straight between the plasma membrane and the callose (Ca). The unit marked by an arrowhead has been cut transversally and shows a circular outline with a dark core and light outer zone; the total diameter is ca. 100 nm. Fixation: RR. Bar=0.5 μm (65,000X). Fig. 7. Early tetrad stage showing aperture sites (arrows). The plasma membrane becomes invaginated between aperture sites and its profile becomes angular with the apex of each angle at the aperture site. Callose (Ca), nucleus (N) and nucleolus (star). Fixation: RR. Bar=10 μm (8,000X). Fig. 8. Detail of a future aperture at the stage shown in Fig. 7. The outer lamellation of the plasma membrane has a thin darkly contrasted coating of glycocalyx (between arrowheads; see Fig. 28). Fixation: RR. Bar=0.5 μm (50,00X).

At the margin of the future apertures the glycocalyx matrix tapers from ca. 50 nm to about the height of interapertural procolumellae (Fig. 28).

Elongation (growth) of early procolumellae

An increase in procolumellae height is accompanied by period when units can be seen to be composed of two components (Fig. 9). These units have an outer part (binders) that is only weakly contrasted and is striated ("coiled") and an inner component that is strongly contrasted. The nature of these two components in early exine development is pronounced in oblique sections (Figs. 10, 11). The coils of the exine development is pronounced in oblique sections (Figs. 10, 11). The coils of the binder part of units show well in Fig. 11 as does also the circular profile of the core zone. Irregular extensions of the core are apparent in some sites in both Figs. 10 and 11.

Development of tectum

The earliest definite protectum is in material like Figs. 12 and 13. The protectum is seen in cross sections (Fig. 12) as narrow extensions in contact with callose at the top of units. In oblique sections the tectum is seen as a ring with an uncontrasted core (Fig. 13). Both sides of the ring are seldom seen in TEM sections because the ring is larger than the thickness of the sections. The thick section in Fig. 14 gives information about the tectum.

At this early stage there is the beginning of a nexine component (Figs. 12, 14). We have not determined whether it is foot layer or endexine or both (see section on formation of the foot layer and endexine).

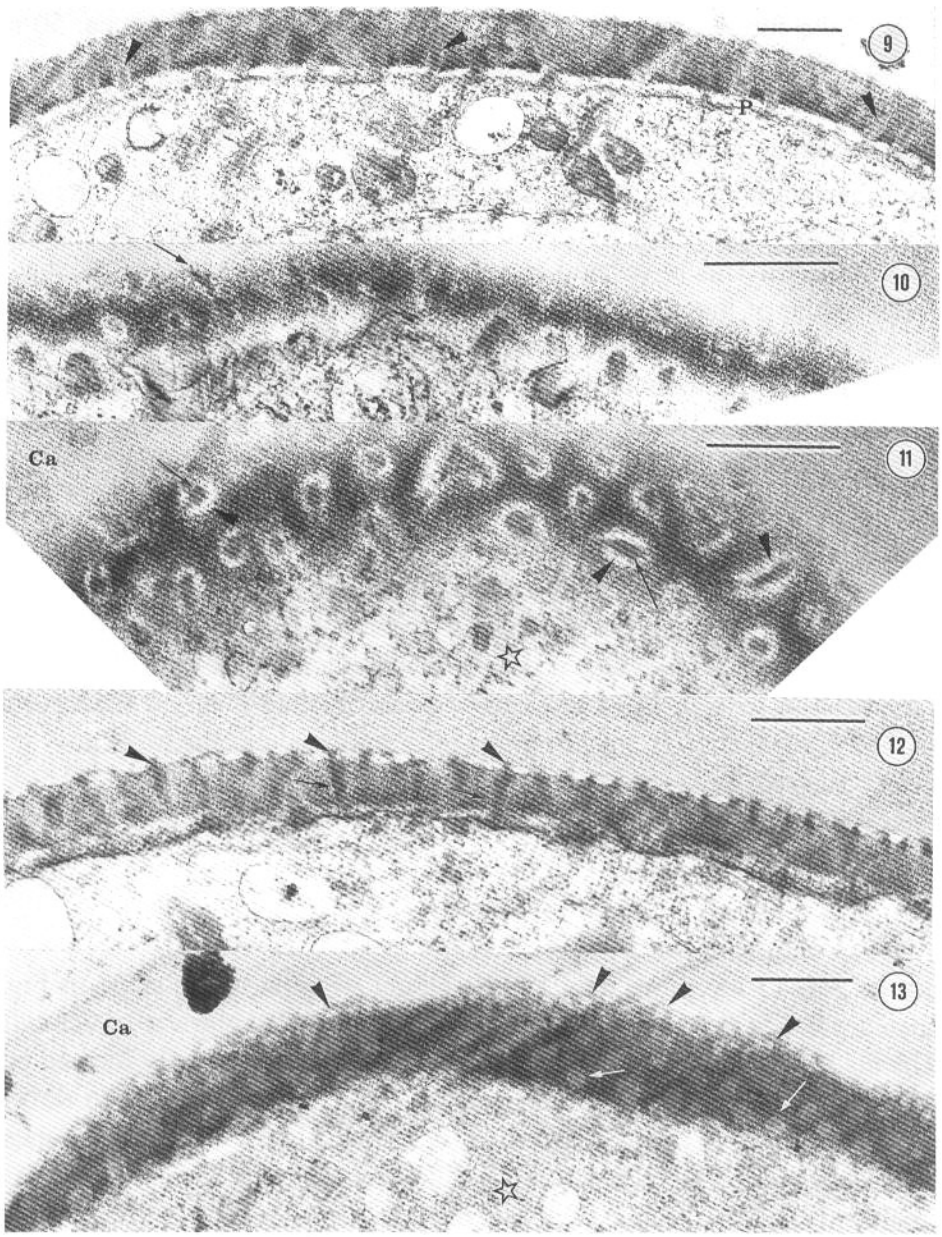
Great increase in height of units

The units in Fig. 15 are more than X2 the height of units in the stage in Fig. 12. These procolumellae are 0.4 to 0.5 μm in height from the plasma membrane to the callose special cell wall. The distal part has expanded laterally while the more recently formed part nearer to the plasma membrane has the 70-100 nm width of units formed earlier in development. In some planes of section these columellae are cone shaped (Fig. 15). In other planes of section, or slightly older stages, columellae show urn-like shapes which are large in the top portion and small below (Fig. 16). Some of these early columellae widen (spread out) near the plasma membrane (Fig. 16). This may be a foot layer development. In oblique and tangential section the difference in size is clear between the units at the level of the tectum and the cell surface (Figs. 17, 18). These planes of section also show that the wall of columellae is continuous and circular.

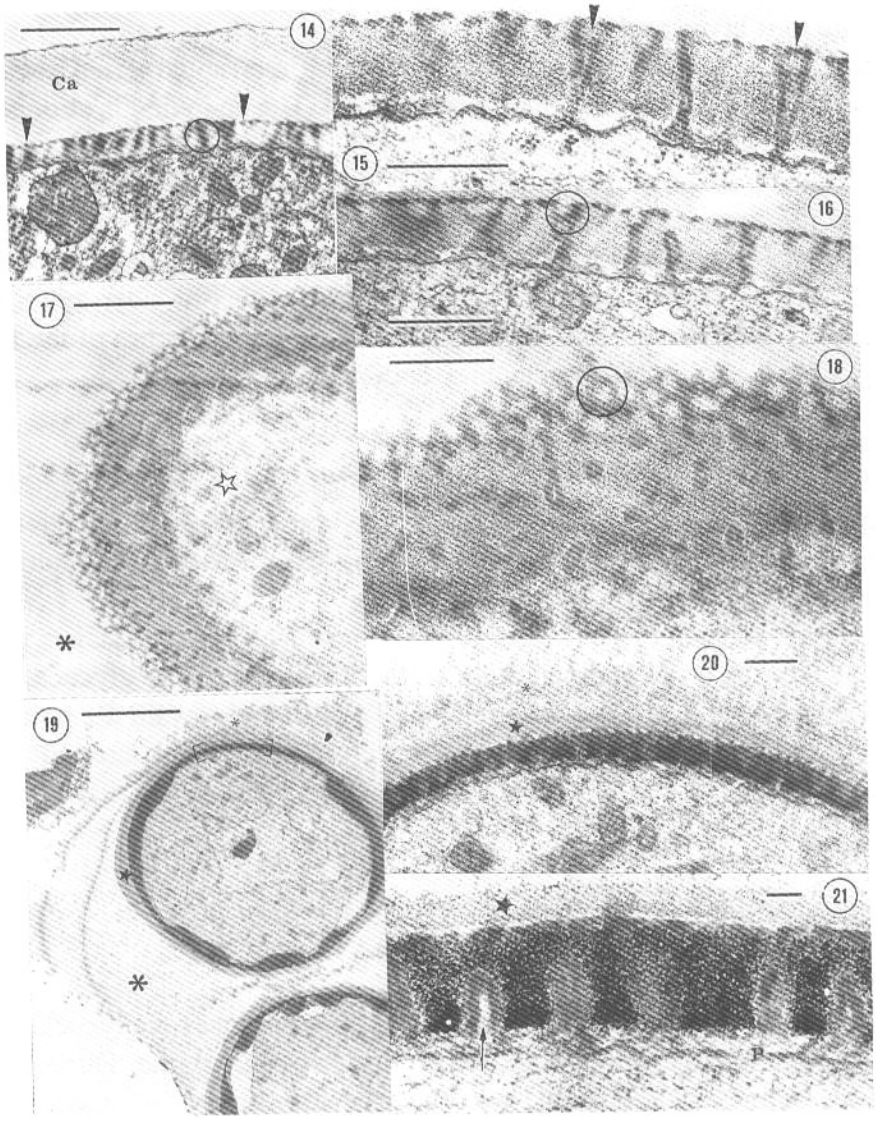
The core zone is low in contrast during this stage in contrast to stages represented in Figs. 3-14.

Loss of callose in MMC envelope

Columellae become more robust and parallel after loss of the outer callose that enveloped the entire tetrad (Figs. 19-21). Contacts between the callose of the special cell wall and the tectal part of procolumellae are apparent in thick and strongly contrasted sections (Fig. 20). The basal widening of columellae that may be initiation of the foot layer is seen in Fig. 21.



Figs. 9-13. Mid-tetrad stages. A mid-tetrad stage of microspore development. In Fig. 9 glycocalyx units (procolumellae, arrowheads) are in contact with the plasma membrane (P). The height of the units is 2-3 times greater than in the early tetrad stages (e.g., Fig. 4). There is little or no tectum as can be seen in the slightly oblique section in Fig. 10 and strongly oblique sections in Fig. 11. When tectal components are initiated they outline polygons, as in Figs. 13, 17 and 18. The oblique sections in Figs. 10 and 11 emphasize the contrast difference between the dark central (core, arrows) part of glycocalyx units (procolumellae) and the light peripheral (binder, arrowheads) part. The core-binder contrast differences at this stage are apparent in Fig. 9 but it is greatly accentuated in the oblique sections. It is the binder portion of these glycocalyx units that shows light and dark striations (Fig. 11, arrowheads). Fixation: RR. Fig. 9 Bar=0.5 μm (28,000X); Figs. 10-11 Bars=0.5 μm (45,000X). Figs. 12 and 13. Initiation of the tectum during mid-tetrad stages. The procolumellae (arrows) elongate distally over the primexine matrix. The elevated portions of units are marked by arrowheads in Fig. 12. The tectal components which appear as isolated extensions of units in thin section, are actually connected to neighboring units. In the tangential section in Fig. 13 the tectal portions of units (at the exine surface) are seen to be polygonal (arrowheads). Fixation: Fig. 12 = AB; Fig. 13 = LN. Fig. 12 Bar=0.5 μm (39,000X); Fig. 13 Bar=0.5 μm (34,000X).



Figs. 14-21. Early tectum of mid-tetrad stages. The section in Fig. 14 is thick enough (ca. 100 nm) to contain a major portion of the glycocalyx units --procolumellae (one is circled). The tectal "ends" (arrowhead) of units appear as a row of beads. Figs. 15 and 16 show sections almost longitudinal (sections are almost radial). As a result, several procolumellae, with their core zones low-contrasted and binder zones high-contrasted, look like funnels (15, arrowheads; 16, circled). In Fig. 17 an asterisk marks the callose and a star the microspore cytoplasm. The polygonal form of tectum is shown in the oblique sections in Figs. 17 and 18. One tectal polygon is circled in Fig. 18. Below the tectum all units in Figs. 17 and 18 show a core zone which has not yet expanded to the size in the tectum or the size later in development (e.g., Fig. 21). Fixation: Figs. 14-16, RR; Figs. 17 and 18, AB. Fig. 14 Bar=20 μm (4,500X); Fig. 15 Bar=0.5 μm (46,000X); Fig. 16 Bar=0.5 μm (39,000X); Fig. 17 Bar=1 μm (18,000X); Fig. 18 Bar=0.5 μm (40,000X). Fig. 19. There is a partial digestion of the microspore mother cell callosic envelope (asterisks) but the callose of the special cell wall (star) is retained. The thick dark sites are material of the glycocalyx which parallel the meridional apertures of the genus. The clear area at the lower right part of the microspore is under an aperture. Fixation: AB. Fig. 19 Bar=20 μm (4,500X). Fig. 20. An enlargement of the developing exine in the interapertural region framed on Fig. 19. Exine development is more advanced than that in Figs. 15 and 16. The glycocalyx is strongly contrasted after Alcian blue fixation. Fig. 20 Bar=0.5 μm (18,000X). Fig. 21. The microspore mother cell callose is absent from this mid-tetrad stage. The callose of the special cell wall is marked by a star. The procolumellae with the core zone (arrow) exposed are about 100 nm in diameter. The plasmalemma is marked "P". Fixation: LN. Fig. 21 Bar=100 nm (61,000X).

Formation of the foot layer and endexine

Early in the formation of an endexine it is thin and interrupted about each 70-100 nm (Fig. 22). The foot layer is also discontinuous (Fig. 22). The endexine is strongly contrasted but the foot layer is weakly contrasted (Figs. 22-25).

Fig. 23 illustrates a stage when the endexine is thin and the microspores, now separated from the tetrad configuration, are still enveloped in the callose special cell wall. The callose special cell wall remains around at least a portion of microspores until the endexine becomes quite thick (>200 nm) between apertures (Fig. 25). The endexine near apertures becomes thickened during this early stage (Figs. 23, 24). The discontinous nature of the thin endexine apparent in Fig. 22 can also be seen in the thick endexine in Fig. 26.

Early development of apertures

The first stages in aperture initiation (Figs. 5, 28) are described in the section on formation of apertures. The glycocalyx tapers to a thin (ca 50 nm) edge in equatorial sections (Fig. 28). The procolumellae are also less in height in the developing aperture margin (not shown well until Fig. 30).

During the period of increase in height of procolumellae (between stages represented in Figs. 12 and 15) there is a dramatic addition to the thickness of the glycocalyx all around apertures. This shown lateral to apertures in Figs. 29 and 30 and toward the pole in Fig. 19.

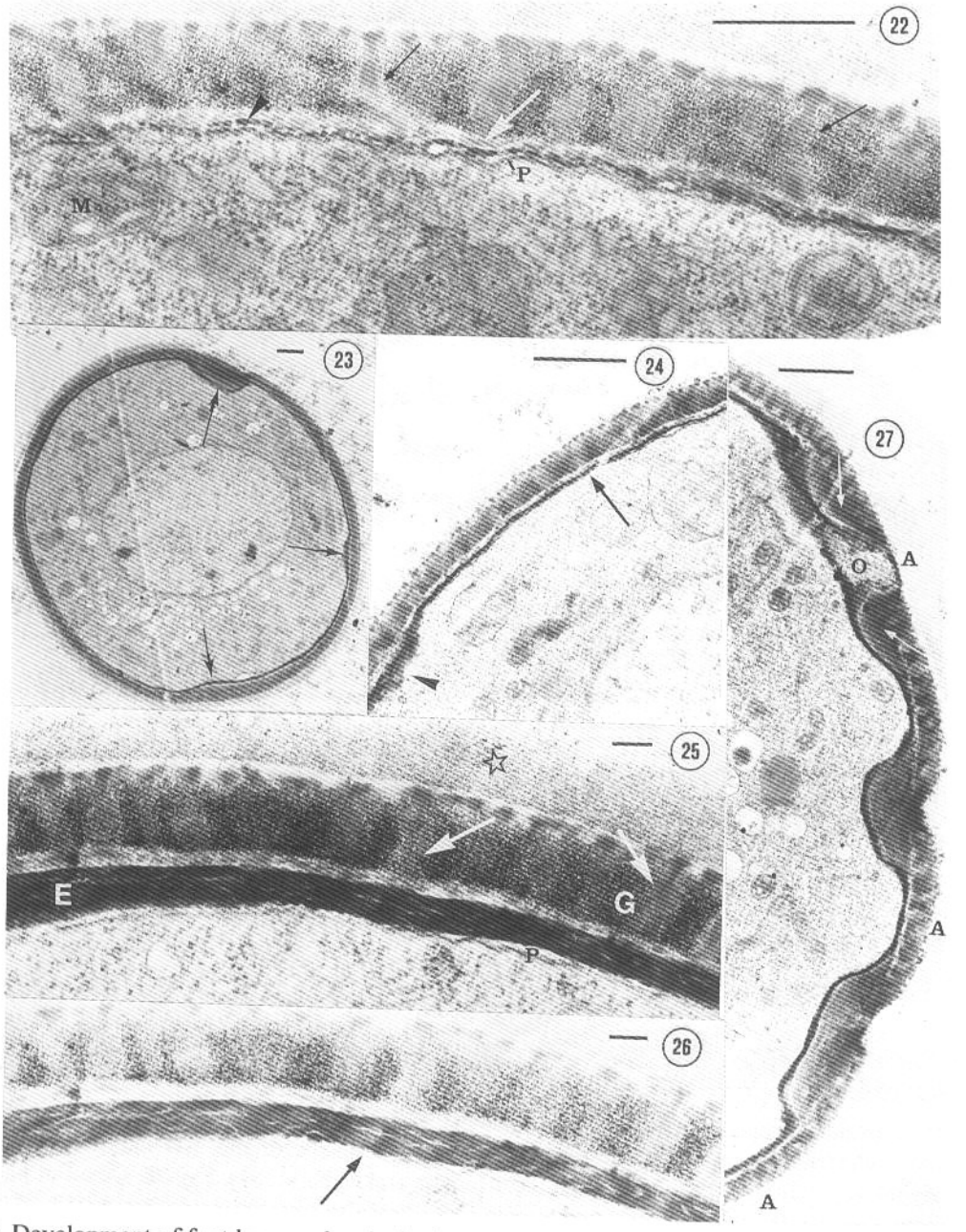
After initiation of the endexine these glycocalyx thickenings around the apertures become prominent (Figs. 23, 27, 31), especially so in Fig. 32, the most mature microspore in this report.

DISCUSSION

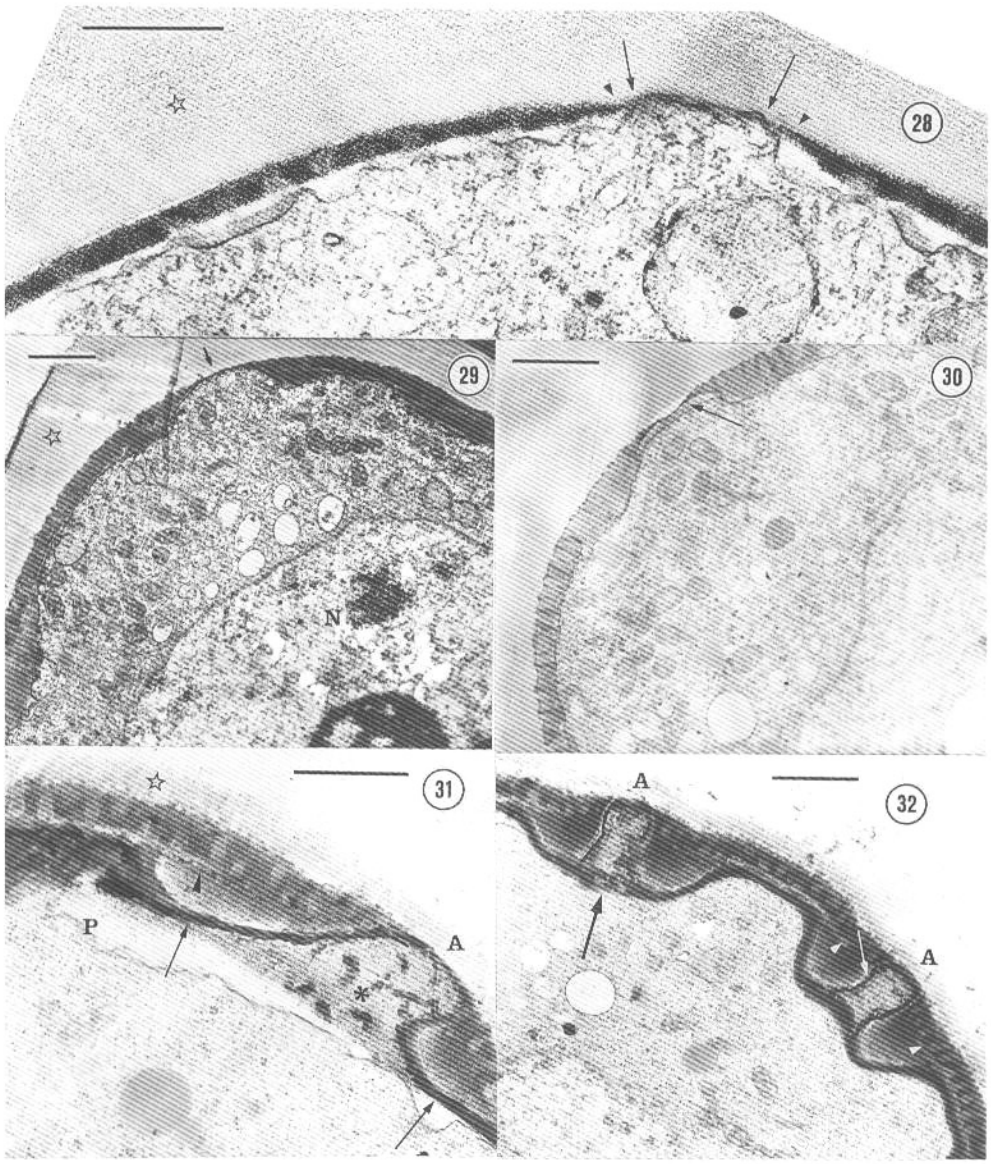
In exine ontogeny of *Borago* the plasma membrane produces a glycocalyx during the tetrad stage which outlines the future aperture and interaperture exine. During the stages of early microspore tetrad development we show evidence for increase in height of procolumellae by growth from the plasma membrane, as is typical for plant cells. When the foot layer and endexine are initiated, however, then there would seem to be an end to outward extension of columellae from the plasma membrane. Subsequent growth of columellae is very great (Ben Saad-Liman and Nabli 1984) and that may assumed to be supratectal.

Outward (supratectal) growth is common in the pollen exine of angiosperms. The classic example of supratectal growth is the work of Takahashi and Kouchi (1988) on formation of spines on the exine of Hibiscus. The foot layer is segmented (interrupted) in the stages of *Borago* microspore development represented here. Micrographs of Ben Saad-Limam and Nabli (1984) show, however, that foot layer components are contiguous between apertures by maturity. Zavada and Wei (1993) described the interrupted foot layer in some species of Camellia (Theaceae) as a "foliated foot layer".

Images of V-shaped columellae are not unusual during exine development in different species. For example Dunbar and Rowley (1984: Fig. 48) interpreted them in *Betula* as oblique sections of cylindrical units. In *Borago* we find, by virtue of the analysis of many sections in different planes, that the units appearing V-shaped in TEM sections are actually



Figs. 22-27. Development of foot layer and endexine interaperturally during mid-tetrad stages. Fig. 22. Initially the foot layer and endexine consists of a bead-like file of dark contrasting segments (arrowhead) adjacent to the plasma membrane (P). The foot layer (white arrow) is also discontinuous but weakly contrasted. Columellae (black arrows) are cut obliquely and not continuous in the figure. Fixation: LN. Bar=0.5 μm (46,000X). Fig. 23. Section passing by the polar ends of three apertures (arrows). The endexine is thinnest between apertures. Fixation: LM. Bar=1 μm (4,500X). Fig. 24. The next step beyond the stage in Fig. 21 is the development of an endexine consisting of several lamellations (arrow). The thickening at the left is near an aperture (arrowhead). Fixation: LN. Bar=1 μm (16,000X). Fig. 25 and 26. These are two prints of the same negative. Fig. 25 is dark to show the callosic special cell wall (star) and plasma membrane (P) below the dark endexine (E),glycocalyx (G), and procolumellae (arrows). Fig. 26 was printed light in order to show the complexity of the endexine (arrow). Fixation: LN. Bar=0.1 mm (65,000X). Fig. 27. Section through different levels of three aperture regions (A). Only the top most of the three includes the aperture proper with its oncus (O). The section emphasizes the glycocalyx thickenings (arrows) at either side of apertures and at their polar ends (the two lower apertures). Fixation: LN. Bar=1 mm (13,000X).



Figs. 28-32. Sections of apertures in the equatorial plane from an early tetrad stage through to endexine formation after the enveloping MMC callose is digested. For details of the 3-D nature of these complex apertures see micrographs and drawings of Ben Saad-Limam and Nabli (1984). Fig. 28. Detail of plasmalemma over an apertural region (between arrows) and thinning of glycocalyx over the extensive margin of the aperture (arrowheads). At this stage the plasma membrane is not invaginated at aperture sites where there is no primexine formation. Callose (star). Fixation: RR. Bar=0.5 μm (39,000X). Fig. 29. An aperture in an early mid-tetrad stage before tectal formation. The cytoplasm protrudes as above and impinges upon the apertural "membrane" (arrow). Callose (star). Fixation: LN. Bar=1 μm (9,200X). Fig. 30. An aperture during a late mid-tetrad stage where there is a tectum. There is an oncus (arrow) between the apertural "membrane" and the plasma membrane. Callose (star). Fixation: AB. Bar=1 μm (12,500X). Fig. 31. The MMC callose has been digested but the special cell wall callose remains (star). The endexine is indicated by arrows. The central part of the apertural zone (A) is bordered and crossed by components of the endexine (asterisk). The foot layer extends into the thickened glycocalyx (arrowhead). The endexine does not cross under the aperture zone in the equatorial region. Fixation: LN. Bar=1 μm (15,000X). Fig. 32. These two apertures (A) are sectioned somewhat poleward from the equatorial plane. The foot layer extends under columellae at either side of apertures (arrowheads). The endexine extends across the proximal part of the apertural zone (arrow) as well as bordering its sides (white arrow) and distal portion. Fixation: LN. Bar=1 μm (11,300X).

funnel or cone-shaped. We have recorded columellar growth that became 100 to 150 nm distally near the callose in conjunction with newly formed basal portions near the plasma membrane that are only ca. 70 nm in width.

There is emphasis on stain reversal between core and binders of units in our work on the early development of the procolumellae of *Borago*. Dunbar and Rowley (1984) called attention to a contrast reversal between core and binder components of procolumellae in *Betula*. Stain reversal is shown for the columellae of *Epilobium* by Rowley and Claugher (1996). Rowley's interpretation is that the staining is strong because the coils of core subunits are located within the core and the contrast weak when the coils of core subunits are interdigitated with binder coils (Rowley 1987-88, 1995).

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摘　　要

玻璃苣小孢子在四分孢子期時，原柱狀層直徑為 70 至 100 nm，具有條狀或螺旋狀的次級構造。網叢具有圓形的中心帶，初期對比度較強，而外環帶對比度較弱。當柱叢高度增加時，其頂端直徑增為 150 至 200 nm，而基部新形成的部分則仍為 70 至 100 nm，此時原柱狀層為圓錐狀。在形狀和高度改變時。柱叢的對比度亦產生逆轉變化，即中心帶對比度變弱而外環帶對比度則變強。外環帶呈條狀或螺旋狀。蓋頂層的形成包含了柱叢頂端的延長與升高，及其對比度強弱的轉變，因此外環帶呈較深的對比度。原柱狀層由原生質膜至胼胝質壁之厚度為 0.4 至 0.5 μm 高。由斜切面觀察，原柱狀層頂端部分的構造交錯而呈多角型。當小孢子母細胞外的胼胝質壁逐漸消失時，蓋頂層變得更顯著，而基層和內層也開始明顯。花粉的萌芽孔在胼胝質壁消失之前變得較複雜，當其形成時，原生質膜和胼胝質壁僅相隔 50 nm，而在這些初形成的萌芽孔邊緣，臘梅糖基質較尖細，高約 50 nm，但是在孔間區域的原柱狀層則有 150 nm 高。胼胝質壁一直持續到小孢子釋出才消失。

關鍵詞：環帶，玻璃苣屬，胼胝質，小孢子，臘梅醣單位，原柱狀層，四分孢子。

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