

## CHAPTER 26

THE OUTER EPIDERMIS OF THE  
COTTONSEED

Jerry D. Berlin  
Texas Tech University  
Lubbock, Texas

## INTRODUCTION

Cotton fibers are single cells that arise from the outer epidermal layer of the cottonseed. Primordial fiber cells initiate elongation on the day of anthesis (Balls, 1915; Aiyangar, 1951; Joshi *et al.*, 1967). The fibers continue to elongate for 16-19 days and then undergo a thickening stage until boll opening. The elongation phase involves primary cell wall synthesis (of course many other things must also be synthesized such as the plasma membrane, internal membranes, cytosol, etc.) whereas the thickening stage is correlated with the synthesis of the secondary cell wall (Balls, 1915). The elongation and thickening stages may overlap (Schubert *et al.*, 1973), and they do not appear to be controlled by the same genetic factors (Kohel *et al.*, 1974).

Characteristics of the fiber primordia at anthesis (flowering) include cell and nuclear enlargement, vacuolarization and the aggregation of smaller nucleoli into a single large nucleolus in the young fiber (Ramsey and Berlin, 1976a,b). There are two types of fibers on most commercial cottons. These are the lint fibers (which are ginned off) and the fuzz fibers that remain on the seed after ginning. The lint fibers initiate elongation on the day of anthesis or shortly thereafter, whereas the fuzz fibers are thought to initiate elongation at later times, e.g., at 4-10 days postanthesis (Barritt, 1929; Aiyangar, 1951; Joshi *et al.*, 1967). Fuzz fibers are distinguished from lint fibers in that they are shorter, have thicker secondary cell walls at maturity, are frequently pigmented differently and, occasionally, are located at specific sites on the cottonseed. The relative numbers of epidermal cells to lint fibers to fuzz fibers varies depending upon the type of cotton.

This review will concentrate on the events surrounding fiber differentiation and initiation in the outer epidermal layer of the cottonseed and will focus on three areas: (1) the morphology of the epidermal cells and the fibers; (2) the incorporation of various radioactive precursors into the epidermal cells and developing fibers; and (3) the seed surface and the number of epidermal cells and fibers on a single seed.

## MORPHOLOGY OF THE OUTER EPIDERMAL LAYER

The morphological descriptions will be presented at the cellular (light microscopy) and subcellular (electron microscopy) levels. All of the light microscopic data were obtained with glutaraldehyde-fixed, plastic-embedded specimens sectioned at 1-2  $\mu\text{m}$ . Numerous stains were employed including aqueous toluidine blue which works well for general morphology. The use of specific cytochemical stains will be mentioned as appropriate. Specimen preparation for electron microscopy was previously described (Ramsey and Berlin, 1976a,b). The plant materials used for most of the morphological work were the commercial cultivars Dunn 56C and Paymaster 266.

### LIGHT MICROSCOPY

Martin *et al.*, (1923) reported differentiation of the floral bud approximately 23 days preanthesis. I examined ovules from 23-day preanthesis squares and found approximately 1,000 outer epidermal cells, each of which was cuboidal and 8-9  $\mu\text{m}$  in length (the preanthesis material used in this study was dated using 3- and 6-day separation for vertical and lateral reproductive buds, respectively, as suggested by McClelland and Neely (1923) and Gore 1932).

Light micrographs of the outer epidermal layer of the developing cotton seed from 4 days preanthesis to 15 days postanthesis are shown in Figures 1-8. At 4 days preanthesis, the outer epidermal cells were generally columnar in shape with dimensions of 9 X 9 X 15  $\mu\text{m}$  (Figure 1). The single nucleus, containing chroma-

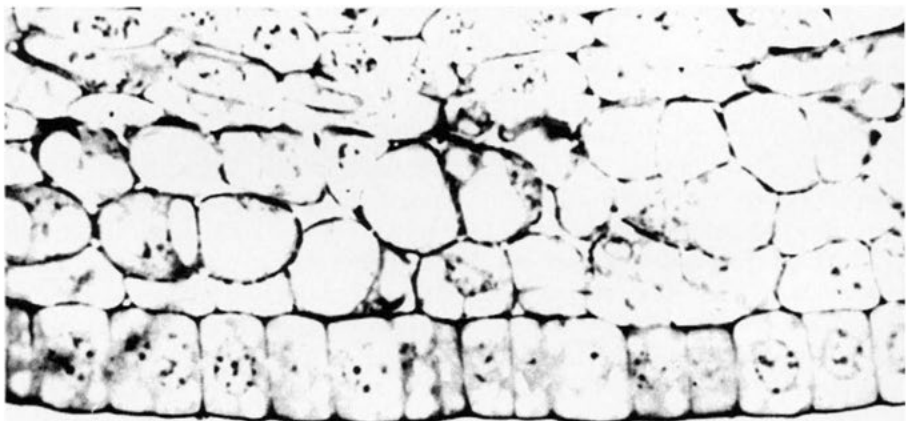


Figure 1. Light micrograph of the outer epidermis of the cotton ovule at 4 days preanthesis. The centrally positioned nuclei each contain peripheral heterochromatin and several small nucleoli. The cytoplasm contains many small vacuoles. (Aqueous toluidine blue stained, 550X magnification).

tin flakes, was centrally positioned. Nucleoli were less than  $1\ \mu\text{m}$  in diameter, and as many as four nucleoli were present in a nucleus. Numerous mitotic figures were apparent in the epidermal layer at all preanthesis times. Numerous small vacuoles were scattered throughout the cytoplasm of the cells. Cells with enlarged nucleoli, presumably fiber primordia, were occasionally observed at three days preanthesis.

At one day preanthesis, the epidermal cells retained their shape, but were

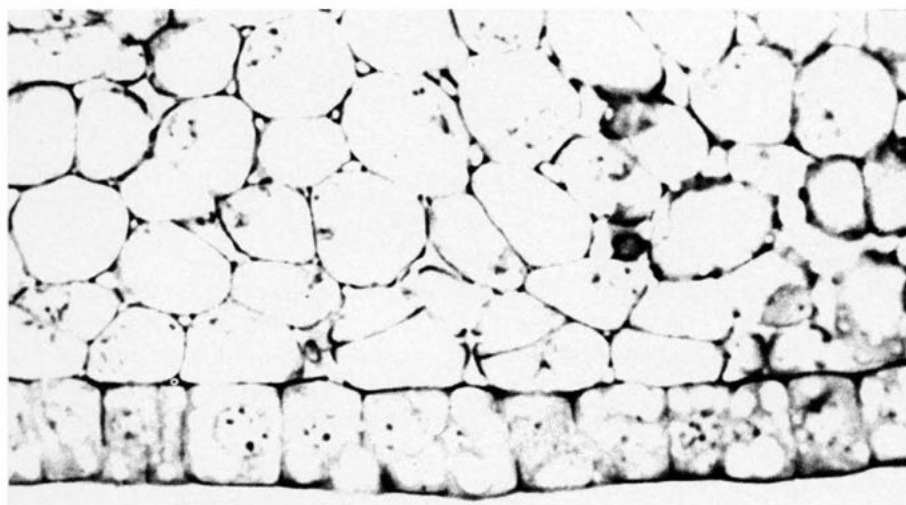


Figure 2. The outer epidermal layer of a cotton ovule at 1 day preanthesis. The nucleoli become prominent and many of the vacuoles have coalesced resulting in fewer, but larger, vacuoles. (Aqueous toluidine blue stained, 550X magnification).

slightly larger (Figure 2). The nuclei were centrally located and exhibited chromatin granules. The nucleus was enlarged in a few cells and a single large nucleolus (up to  $2\ \mu\text{m}$  diameter) was prominent. Cytoplasmic vacuoles appeared to coalesce resulting in fewer, but larger vacuoles. Although not shown in black and white photographs of aqueous toluidine blue stained sections, many of these vacuoles were green to dark green in color indicating the presence of phenolic compounds (Feder and O'Brien, 1968). Epidermal cells from younger ovules lacked similar vacuolar staining.

On the day of anthesis, the first series of fiber primordia started to elongate. These initiating fiber cells bulged outward from the surface of the seed (Figure 3). Enlarged nucleoli (over  $2\ \mu\text{m}$  diameter) were present in the nuclei of these fiber initials, and the nuclei possessed little chromatin staining (Figure 3). At one day postanthesis, the tips of the fiber initials projected about  $15\ \mu\text{m}$  above the

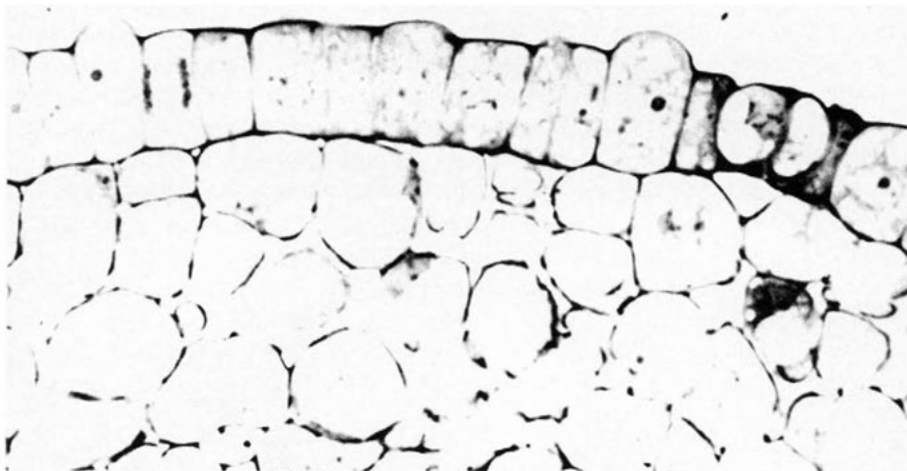


Figure 3. A light micrograph of fiber primordia in the outer epidermal layer of the cotton ovule on the day of anthesis. The fiber primordia, containing prominent nucleoli, have initiated elongation and protrude above the surrounding cells. The vacuoles are less obvious in the fiber primordia than in the neighboring nonfiber cells. Among the nonfiber cells are a cell in anaphase and two guard cells of a stomatal complex. (Aqueous toluidine blue stained, 550X magnification).

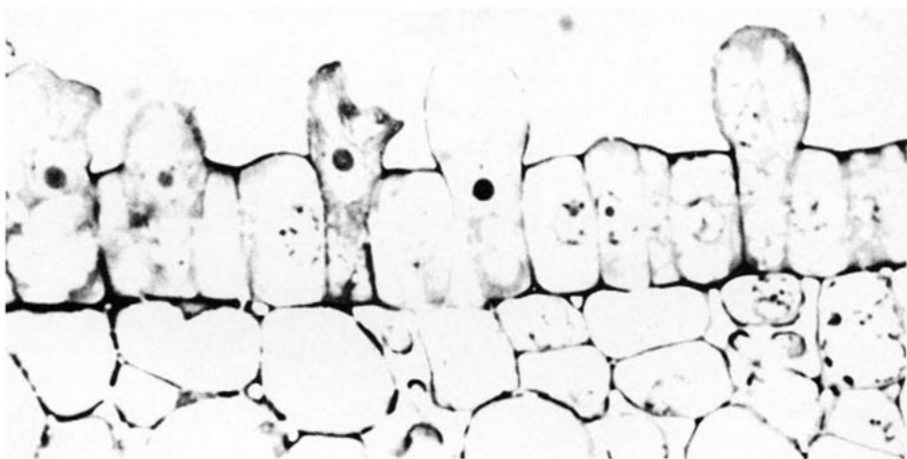


Figure 4. A light micrograph of elongating fibers at 1 day postanthesis. The elongating fibers have a single enlarged nucleolus in a pale staining nucleus that has migrated slightly toward the fiber tip. The cytoplasm contains numerous vacuoles and the large central vacuole has not started to form at this time. (Aqueous toluidine blue stained, 550X magnification).

seed surface (Figures 4, 9), and various cellular structures, including the nucleus, had migrated toward the outer tip of the fiber (Figure 4). The cytoplasm of the young fiber appeared to expand to fill the entire volume of the enlarging cell. The nonfiber epidermal cells maintained their original shape with a slight increase in cell volume, and the nuclei remained centrally located in the cell. There were 50,000 to 60,000 surface cells on the cotton ovule at anthesis. Thus, if all 1,000 epidermal cells in the 23 day preanthesis ovule continued to divide, some 5-6 divisions would be required to arrive at 60,000 epidermal cells at anthesis, and the average generation time would be approximately 5 days.

At 2 days postanthesis the fiber cells that had initiated elongation on the day of anthesis had attained a diameter of about 22  $\mu\text{m}$  and a length of approximately 100  $\mu\text{m}$  (Figure 9). Fiber nuclei were free of chromatin staining and had moved out into the fiber (Figure 5). The single nucleolus in these fibers had enlarged to 4

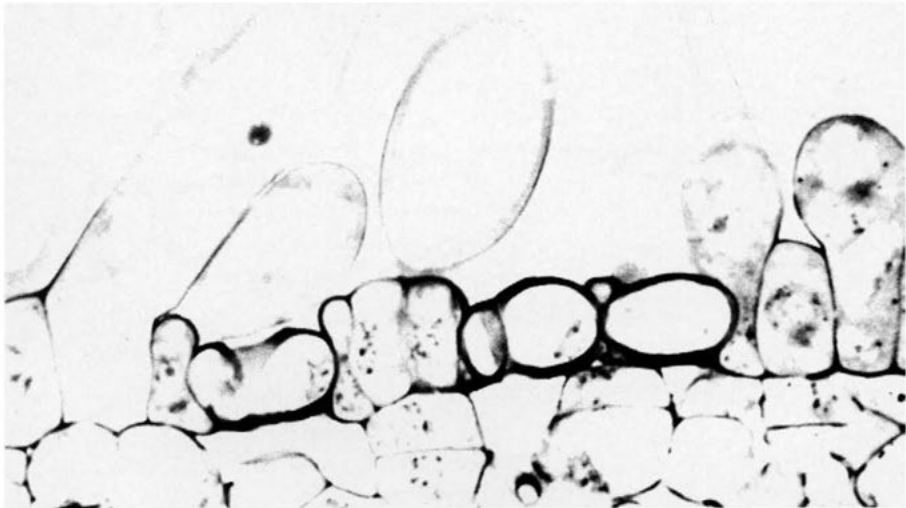


Figure 5. A light micrograph of the epidermal layer and fibers at 2 days postanthesis. Two cells are initiating elongation and several fibers are so large that they are not contained within the field of the picture. A pale-staining nucleus with a large nucleolus is shown in one of the fibers. The older fibers are characterized by large central vacuoles. The cytoplasm of these older fibers contains small vacuoles that may coalesce with the central vacuole. Note the base of the fiber containing the nucleus still has considerable cytoplasm and has not progressed to a "rim" of cytoplasm. Guard cells are shown in the epidermal layer. (Aqueous toluidine blue stained, 550X magnification).

$\mu\text{m}$  in diameter. Other epidermal cells continued to initiate elongation (Figure 5), and the events accompanying initiation in these cells were identical to those previously described to occur at anthesis. The nonfiber epidermal cells were

slightly larger at this time ( $13 \times 13 \times 21 \mu\text{m}$ ). The nuclei were centrally located, and many of the nuclei contained chromatin granules and small nucleoli.

The fiber nucleoli continued to increase in size through 3 days postanthesis (up to  $7 \mu\text{m}$  in diameter). Fiber initiation was not observed between 3 and 5 days postanthesis. The nonfiber epidermal cells enlarged slightly ( $15 \times 15 \times 25 \mu\text{m}$ ), and the nuclei remained centrally positioned (Figure 6). Although mitotic activity was observed in the epidermal layer at this age, it was not as frequent as at preanthesis times. In no case was mitosis observed in these cells after six days postanthesis. The vacuoles continued to decrease in number and increase in size (Figure 6). By 5 days postanthesis the fiber cells attained an average diameter of

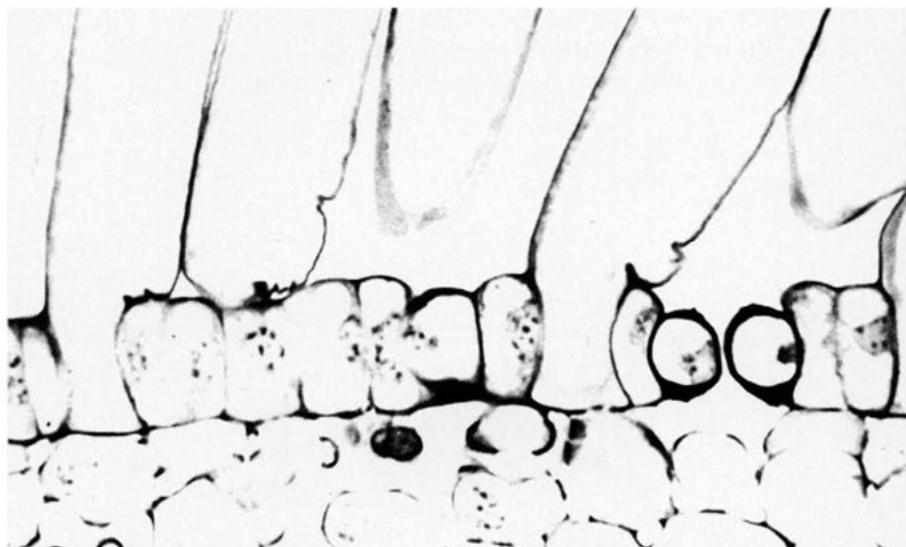


Figure 6. The epidermal layer and fibers at 3 days postanthesis. Each of the fibers has a large central vacuole that extends into the fiber base. The nonfiber epidermal cells contain several large vacuoles. A cross-section of a stomatal complex is shown. (Aqueous toluidine blue stained, 550X magnification).

$29 \mu\text{m}$ , and the longest fibers were 3 mm long (Figure 9).

A new wave of fiber initiation was found between 6 and 8 days postanthesis. Initiating fiber cells, thought to be fuzz fibers, followed the same pattern of initiation as previously described. Between 8 and 10 days postanthesis the non-fiber epidermal cells had a single central vacuole that was filled with phenolic materials (Figure 7). Once the large vacuole was established, further initiation of elongation was never observed.

The vacuoles found in the epidermal cells contained phenolic materials on the day preceding anthesis. These materials were identified as phenolics on the basis

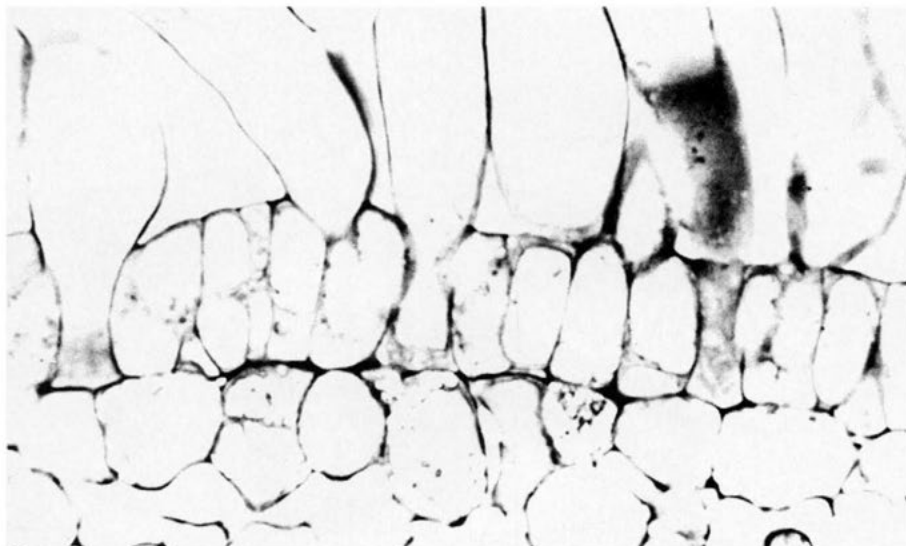


Figure 7. A light micrograph of the epidermal layer at 8 days postanthesis. The fibers have very little cytoplasm in their bases. The vacuoles in the nonfiber epidermal cells have enlarged and are in the process of accumulating phenolic materials. (Aqueous toluidine blue stained, 550X magnification).

of their staining with acid toluidine blue, periodic acid-Schiff, ferric chloride, safranin-fast green and osmic acid (Ginzberg, 1967). These phenolic materials became increasingly condensed, filled the nonfiber epidermal cells (Figure 8) and eventually contributed to the dark coloration characteristic of the cottonseed. At later times, the cells that comprised the seed surface became paradermally enlarged and synthesized thick secondary cell walls.

To summarize our light microscopic studies with respect to the epidermal cells and fiber initiation, nucleolar enlargement was an early indication of fiber primordial differentiation and occurred approximately 2-3 days prior to the initiation of elongation. An enlarged nucleolus is suggestive of increased rRNA synthesis, and thus new ribosomes, which would be correlated with a requirement for new protein synthesis in elongating fibers (Chapter 25). A second event preceding the initiation of elongation was the accumulation of phenolic materials in epidermal cell vacuoles. We shall have more to say about both of these points later. Lint fibers appeared to initiate elongation from anthesis to the second day postanthesis. As previously mentioned, fiber initiation occurs in waves beginning on the day of anthesis (Aiyangar, 1951; Joshi *et al.*, 1967; Lang, 1938). The first wave of initiating fibers become lint fibers (Joshi *et al.*, 1967; Lang, 1938) No initiation of elongation was found between the 3rd and 5th day postanthesis. The second and final stage of fiber initiation was found between 6 and 8 days



Figure 8. The outer epidermal layer of the cottonseed at 15 days postanthesis. The fibers have such a small amount of cytoplasm that it is not visible at this magnification. The nonfiber cells have accumulated massive amounts of phenolic substances in their central vacuoles. (Periodic acid-Schiff stained, 550X magnification).

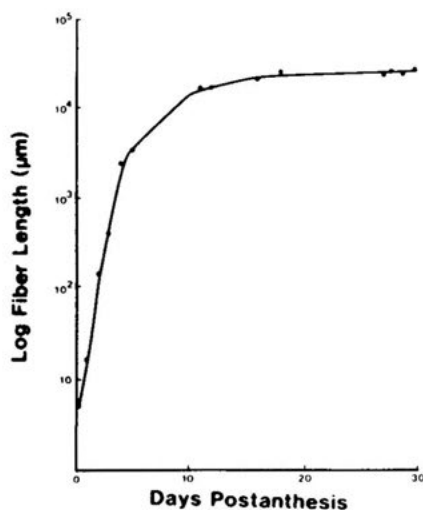


Figure 9. A growth curve of cotton fiber elongation showing increased fiber length with time postanthesis. The log phase of fiber elongation occurs during the first 10 days postanthesis.



postanthesis. These later developing fibers are thought to be fuzz fibers (Joshi *et al.*, 1967; Lang, 1938). The lateral displacement of the nuclei and the concentration of phenolic compounds in the vacuoles of the nonfiber cells at 8-10 days postanthesis caused the internal details of these cells to be obscured at this level of magnification.

### ELECTRON MICROSCOPY

The nuclei and cytoplasm of the epidermal cells from 16 to 3 days preanthesis appeared electron dense and were uniformly dark (Figure 10). The cell wall (primary) at the ovule surface and at the base of the epidermal cells was approximately 150 nm thick; the cell wall located between adjacent epidermal cells was slightly thinner (approximately 80 nm thick). Typical cell organelles such as nuclei, plastids containing starch granules, mitochondria, dictyosomes and vacuoles were observed in the epidermal cells of the ovule. The centrally positioned nuclei were circular to elliptical in shape and contained peripheral chromatin and up to 4 nucleoli. A light nucleolar cap was often observed on the outer edge of one

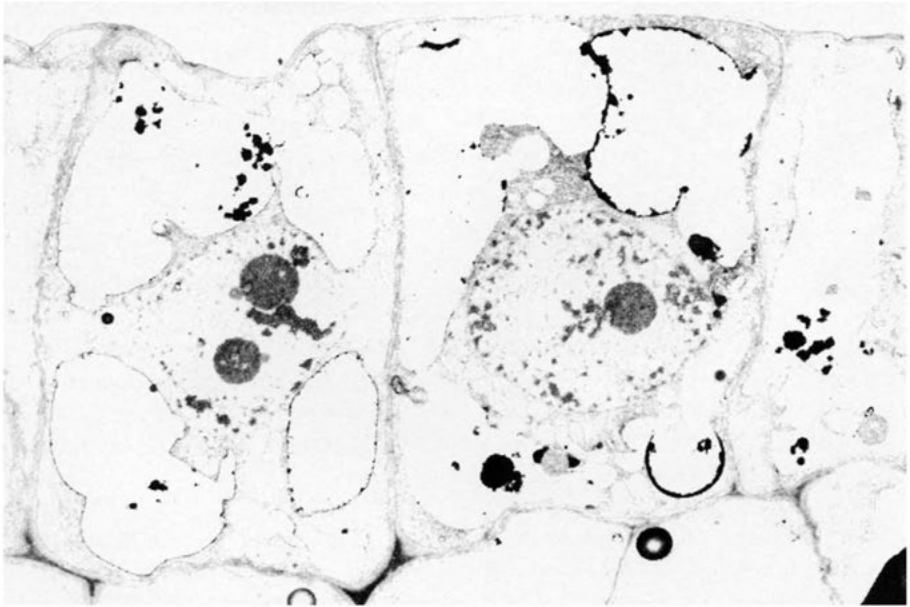


Figure 10. Electron micrograph of a median longitudinal section of the epidermal layer of the cotton ovule at 16 days preanthesis. The cell walls at the free-surface and at the basal region of the cells are approximately twice as thick as the lateral cell walls. The centrally positioned nucleus contains peripheral chromatin and numerous (up to four) nucleoli. Large vacuoles containing a small amount of phenolic material occupy most of the cytoplasm (9,100X magnification). (From Ramsey and Berlin, 1976a).

or more of the nucleoli. The dictyosomes possessed 4 to 7 cisternae and often had an electron dense cisterum at the mature face. Large vacuoles with a small amount of dense pigment were apparent in 16 day preanthesis epidermal cells (Figure 10). The vacuoles at 10 to 3 days preanthesis were smaller and more numerous (Figure 11) than in younger ovules.

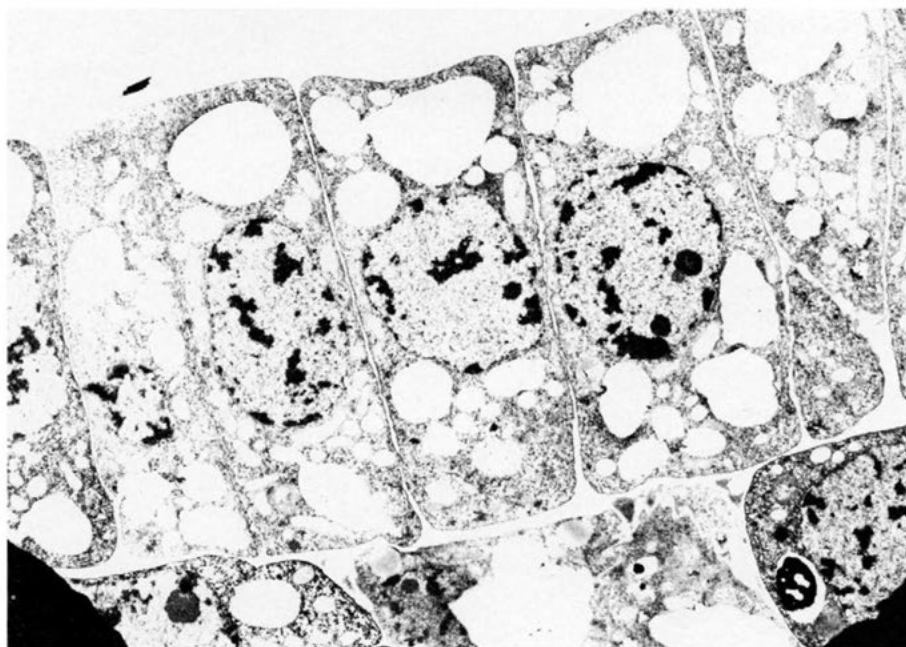
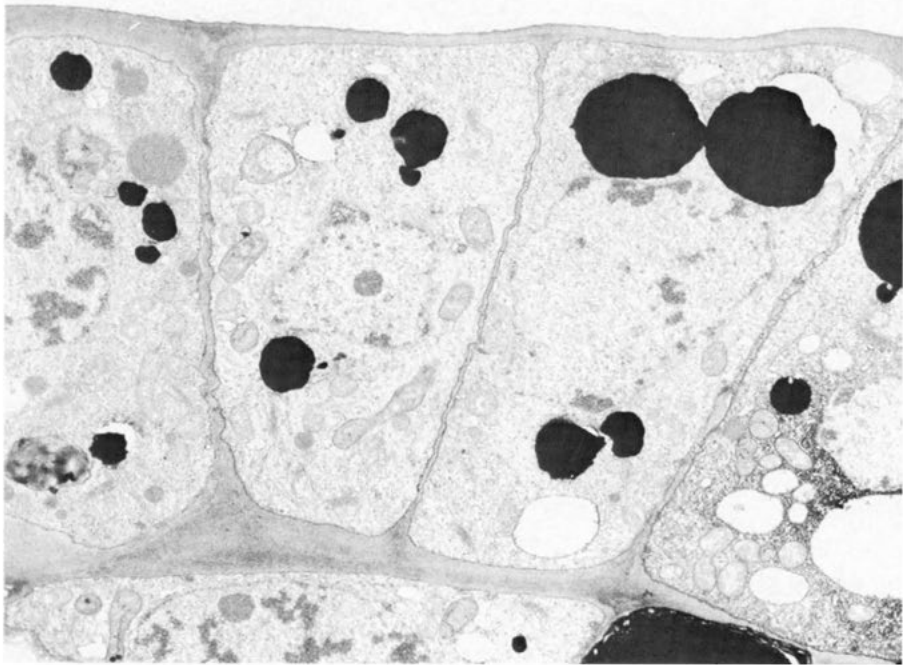


Figure 11. Transmission electron micrograph of the epidermal layer of a cotton ovule at 3 days preanthesis. The centrally positioned nuclei are surrounded by numerous small electron transparent vacuoles. Numerous mitochondria and proplastids are present in the cytoplasm. (8,200X magnification). (From Ramsey and Berlin, 1976a).

The appearance of the cells in the epidermal layer of the ovule was greatly altered approximately 24 hours preanthesis when many of the vacuoles became filled with an electron dense pigment (Figure 12). The nucleus and cytoplasm were conspicuously less electron dense and appeared much lighter than the dense, uniformly dark, epidermal cells of earlier times. The electron dense material in the vacuoles of the epidermal cells was concluded to be pigment as a result of the light microscopic cytochemical staining previously mentioned and the fact that the electron dense material was not observed in the vacuoles following methanol treatment of the ovules which would extract phenolic compounds (Mabry *et al.*, 1970).



**Figure 12.** Median longitudinal section of the outer epidermal layer of a cotton ovule at 1 day preanthesis. Many of the vacuoles are filled with phenolic substances. The nuclei and cytoplasm are less electron dense than in younger epidermal cells. A number of mitochondria, proplastids and dictyosomes are evident in the epidermal cytoplasm. Numerous plasmodesmata occur in the thin lateral cell walls between epidermal cells. (12,600X magnification). (From Ramsey and Berlin, 1976a).

Differentiation of primordial cells (epidermal cells that ultimately produce cotton fibers) in the epidermal layer first became apparent via electron microscopy at 16 hours preanthesis. A few cells near the chalazal end of the ovule were slightly enlarged and had dark nuclei, dark cytoplasm and vacuoles with very little pigment remaining (Figure 13). The cytoplasmic density of these differentiating primordial cells was not as uniform as the cytoplasmic density observed in epidermal cells from 16 to 3 days preanthesis. The cytoplasm immediately surrounding the vacuoles was more electron dense than the cytoplasm further removed from the vacuoles (Figure 14).

Light cells and dark differentiating primordial cells could be observed in the epidermal layer from the chalazal end midway to the micropylar end of the ovule by 8 hours preanthesis. The pigment in the vacuoles of the light cells at 8 hours preanthesis was not as compact nor as uniformly electron dense as the pigment

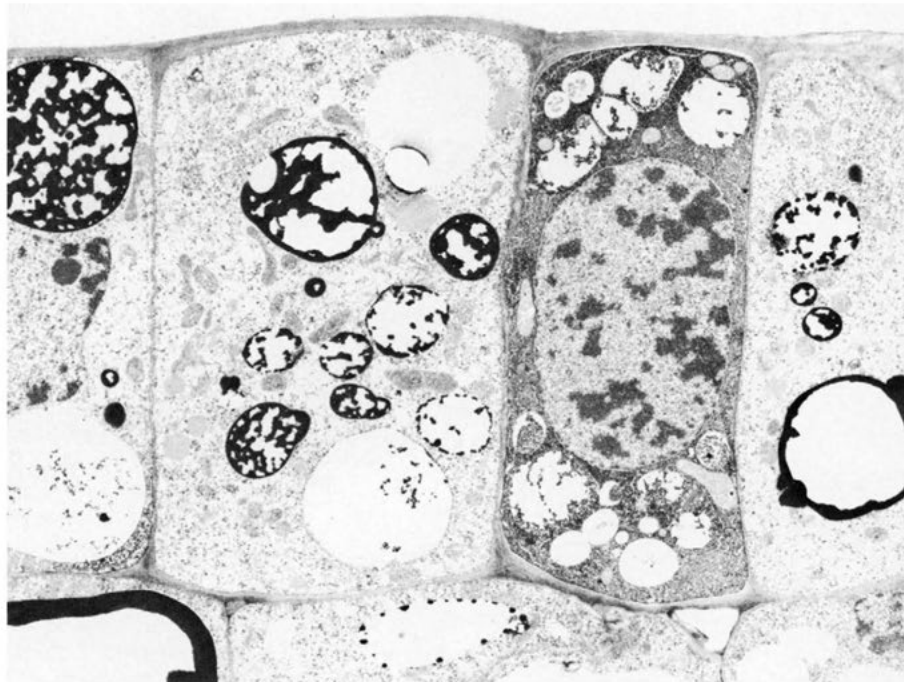


Figure 13. Median longitudinal section of the epidermal layer of the cotton ovule the evening before anthesis. The dark fiber primordial cells have little phenolic material remaining in the vacuoles compared with the light nonfiber cells that have phenolic materials localized within the vacuoles. The dark cells have enlarged nuclei. (10,000X magnification). (From Ramsey and Berlin, 1976a).

found in vacuoles of light cells at 24 hours preanthesis. Comparison of light and dark cells at anthesis revealed that the ribosomes in the cytoplasm of the dark cell were more numerous than the ribosomes in the cytoplasm of the light cell (Figure 15).

Only dark cells with most, but not necessarily all, of the pigment released from the vacuoles were observed to initiate fiber elongation at anthesis (Figure 16). Endoplasmic reticulum and dictyosomal membranes were more prominent in these fiber primordia than in neighboring nonfiber cells at anthesis (Figures 16, 17). The increase in these structures is undoubtedly in preparation for the increased requirement for membranes during the elongation phase of fiber development. The nucleus of the elongating fiber was enlarged at anthesis, had a single enlarged nucleolus, contained very little condensed peripheral chromatin and migrated from its original central position toward the fiber tip (Figure 18). Mitotic activity occurred in adjacent cells in the epidermal layer simultaneously

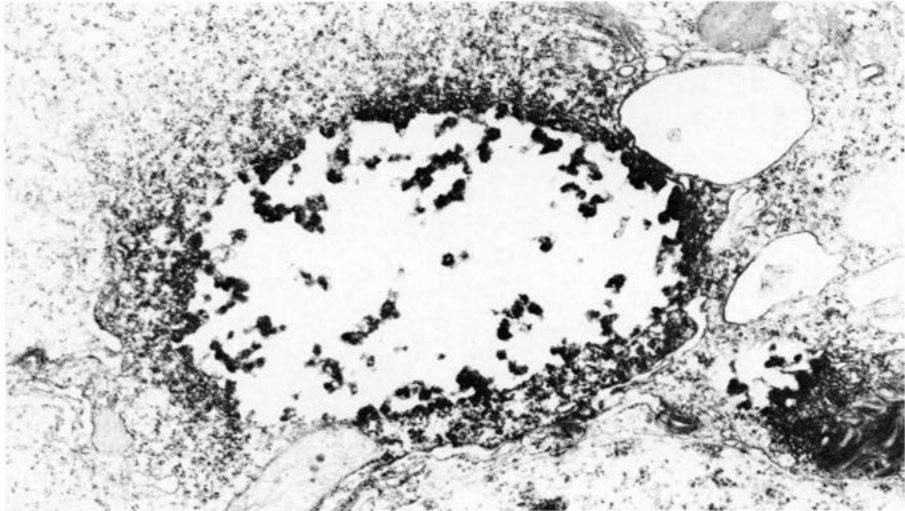


Figure 14. A portion of a fiber primordium showing an early stage in the dispersion of phenolic substances from a vacuole just prior to elongation. Membranes near the vacuole are coated with the phenolic materials, whereas structures further removed from the vacuole are not coated with the electron opaque material. (45,000X magnification).

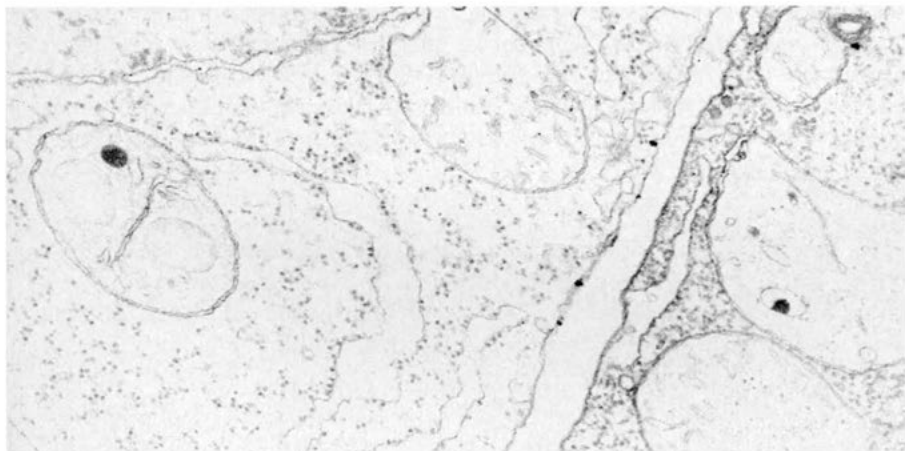


Figure 15. A comparison of the cytoplasm of light and dark epidermal cells. The dark cell contains many more ribosomes than does the light cell. Evidence of phenolic materials adhering to membranes is suggested by the apparent increased thickness of the plasma membrane and the endoplasmic reticulum of the dark cell. (39,000X magnification). (From Ramsey and Berlin, 1976a).

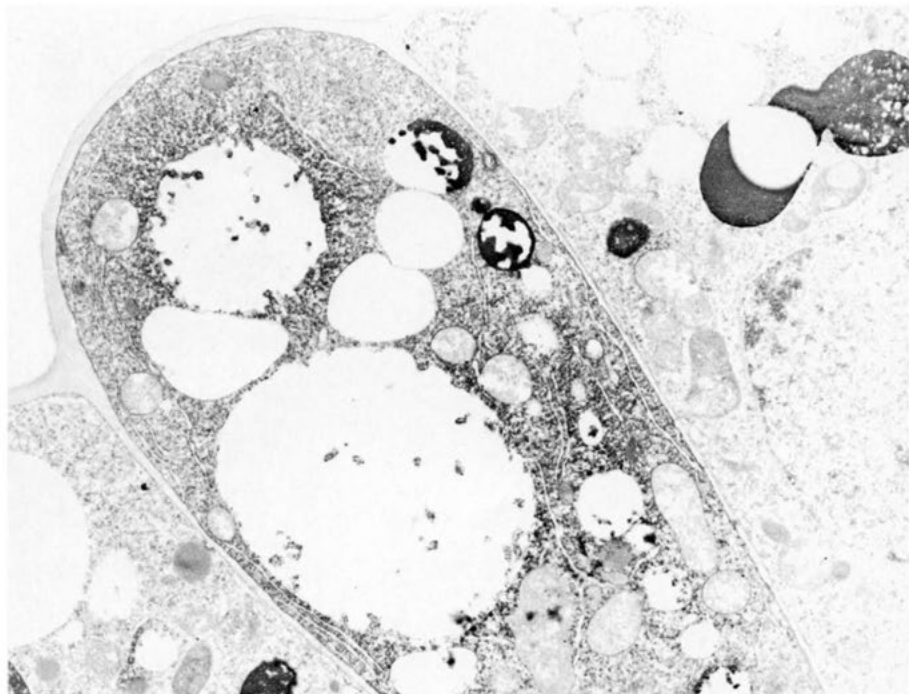


Figure 16. A fiber primordial cell initiating elongation on the day of anthesis. The dark cell protudes above the neighboring nonfiber, epidermal cells. The endoplasmic reticulum of the fiber primordium is well-developed and more extensive than in the adjacent light cells. The vacuoles in the dark cell contain only a small amount of phenolic materials in the vacuoles. (14,000X magnification). (From Ramsey and Berlin, 1976a).

with the initiation of elongation in fiber primordia. The cytoplasm of the elongating fibers became less electron dense first at the fiber tip. (Figure 18); however, the fiber base became less electron dense as the fiber further enlarged. Initiation of fiber elongation from epidermal cells appeared to occur randomly. Two or more fibers were often observed to initiate from adjacent epidermal cells; however, the relationship of the two cells to each other could not be determined. Numerous plasma membrane-associated vesicles were frequently observed in the tips and sides of elongating fibers (Figure 17). These plasma membrane-associated vesicles were not observed in fiber bases nor in nonelongating epidermal cells.

The elongating fibers at one day postanthesis were longer than fibers found at anthesis and appeared to have an increased number of lipid bodies present in the cytoplasm (Figure 19). The formation of the large central vacuole, characteristic of cotton fibers, was apparent by one day postanthesis. Two events led to the

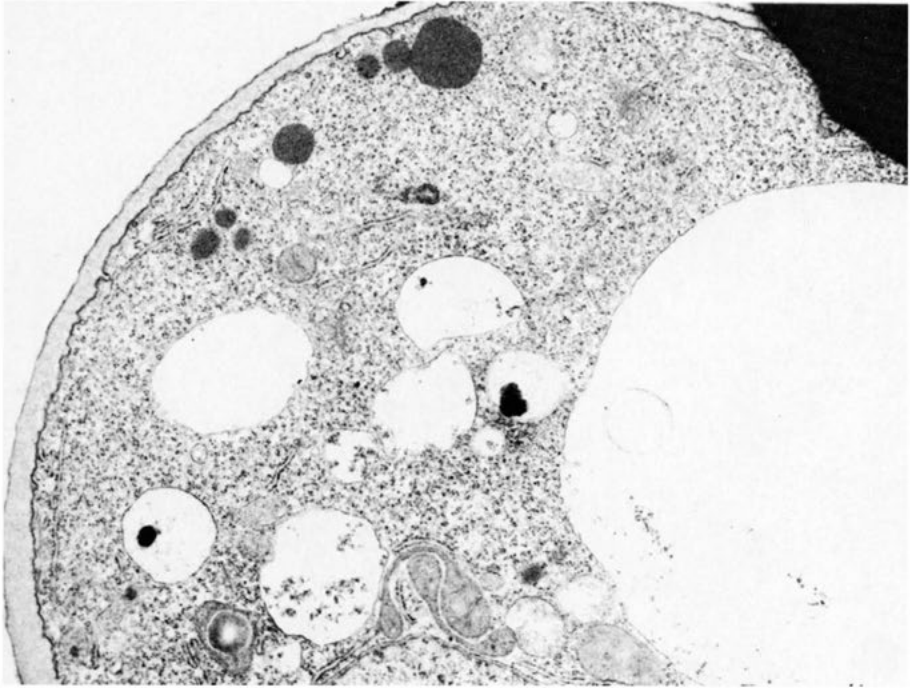


Figure 17. The tip of an elongating fiber on the day of anthesis. The cytoplasm is rich in ribosomes, endoplasmic reticulum and dictyosomes. Numerous plasma membrane-associated vesicles are apparent. (21,000X magnification).

formation of the central vacuole. First, dilation of the endoplasmic reticulum in close association with vacuoles produced a number of small vacuoles (Figure 20). Secondly, the several small vacuoles present in the fiber primordia on the morning of anthesis, as well as the vacuoles formed from the endoplasmic reticulum, coalesced into a larger vacuole. The vacuole, at one day postanthesis, further enlarged and migrated from the basal region into the mid-region of the fiber. The basal portion of the central vacuole at one day postanthesis was approximately even with the surface of the epidermal layer of the ovule and contained a small amount of diffuse pigment (Figure 19). The central vacuole enlarged and extended into the fiber base at 2 days postanthesis, leaving a thin rim of cytoplasm adjacent to the cell wall (Figure 21). A further suggestion of small vacuoles fusing with the tonoplast of the large central vacuole is shown in Figure 21.

The fiber nucleus at one day postanthesis was further enlarged, contained a small amount of condensed peripheral chromatin and had migrated into the mid-region of the fiber. At 2 days postanthesis, the nucleus was irregularly shaped, lacked peripheral chromatin and had a huge "bullseye" nucleolus (Figure 22). A

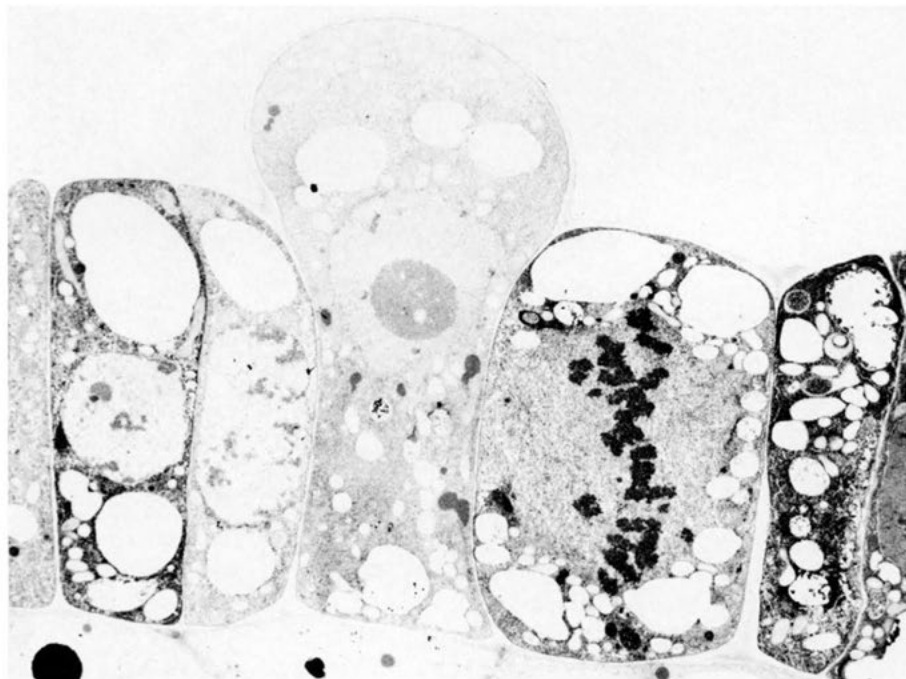


Figure 18. A median longitudinal section of the epidermal layer of the cotton ovule showing a lint fiber tip protruding above the epidermal layer on the day of anthesis. The nucleus of the fiber cell is enlarged, has a single, large nucleolus and has migrated toward the fiber tip. The fiber cytoplasm contains numerous vacuoles and is darker at the base than at the tip. An epidermal cell in mitotic metaphase is shown next to the fiber. (6,000X magnification). (From Ramsey and Berlin, 1976a).

small circular nucleolar cap was routinely observed at the outer edge of the nucleolus. The fiber nucleus remained structurally intact during the later stages of development and was observed in the distal one-third of the fiber at 16 days (Figure 23) and at 37 days postanthesis (Figure 24). The nucleus at these times was irregularly shaped and lacked peripheral chromatin. The fiber nucleolus became smaller after 3 days postanthesis and apparently disappeared by about 6 days postanthesis (however, see Chapter 25).

The cytoplasm in the young fiber tip at 2 days postanthesis contained numerous dictyosomes, lipid bodies, endoplasmic reticulum, plastids, mitochondria and small vacuoles (Ramsey and Berlin, 1976a). Dictyosome-derived vesicles contained fibrils which were similar in morphology to the fibrils found in the primary cell wall (Figure 25). Similarities between dictyosome-associated vesicles and plasma membrane-associated vesicles were apparent, suggesting the former be-



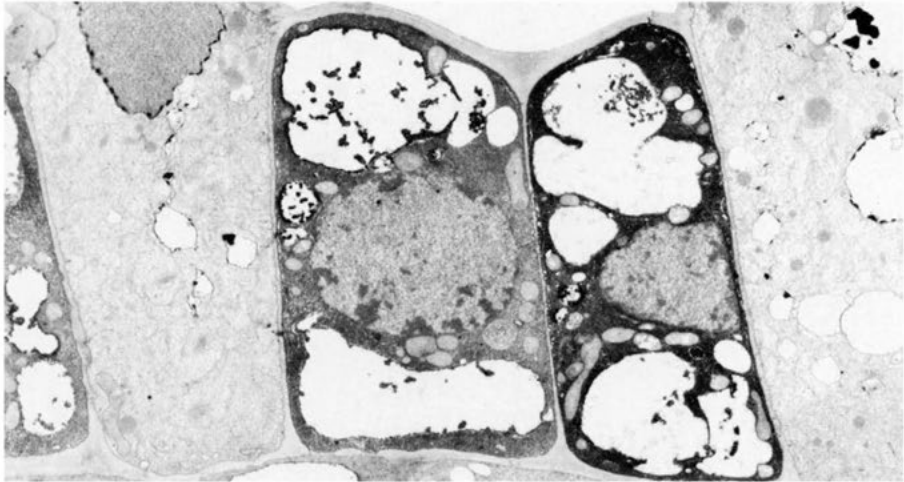


Figure 19. Two elongating lint fibers at 1 day postanthesis. The central vacuole has formed in the fibers above the seed surface. An accumulation of pigment material is characteristically found at the base of the enlarging central vacuole. (4,500X magnification). (From Ramsey and Berlin, 1976b).

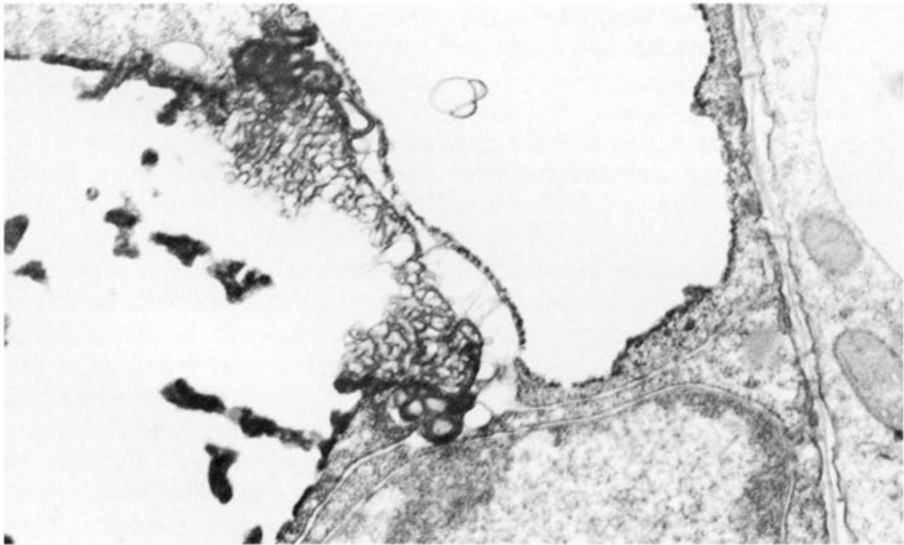


Figure 20. A portion of a dark cell on the day of anthesis with endoplasmic reticulum cisternae continuous with a fenestrated membranous network associated with a vacuole. The endoplasmic reticulum also shows continuity with the nuclear envelope. (44,000X magnification). (From Ramsey and Berlin, 1976a).

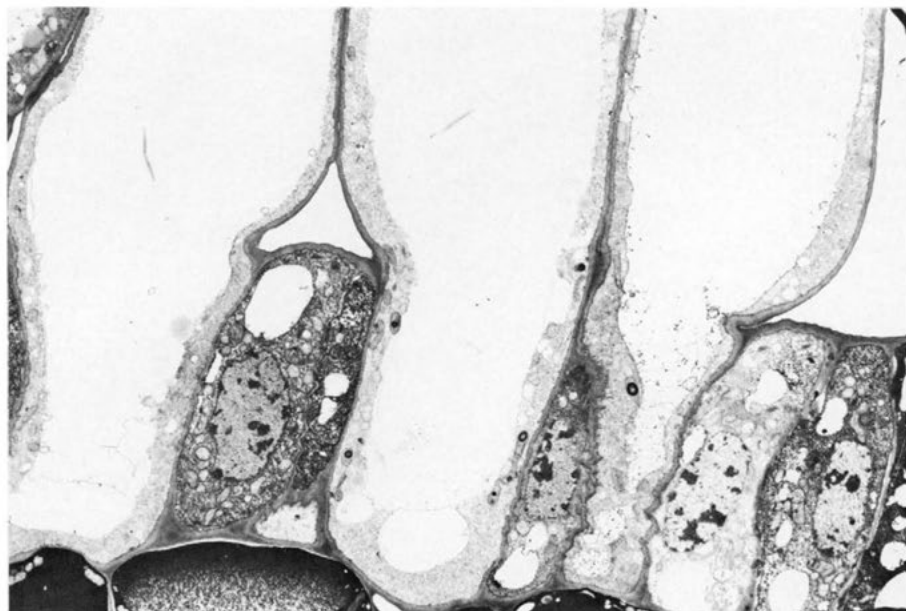


Figure 21. Cotton fibers at 2 days postanthesis have central vacuoles extending into the fiber bases, leaving a thin rim of cytoplasm around the central vacuole. Several small cytoplasmic vacuoles appear to be in the process of fusing with the tonoplast of the central vacuole. (5,000X magnification). (From Ramsey and Berlin, 1976b).

came the latter via an exocytotic secretory process (Ramsey and Berlin, 1976b; Westafer and Brown, 1976). Dictyosome-associated vesicles found in fibers during secondary cell wall thickening did not contain fibrils characteristically found in dictyosome-associated vesicles during primary cell wall synthesis.

Most of the ribosomes in the young fiber were free and unattached to membranes (Figure 25) and only a few were in the polysome configuration during primary cell wall synthesis. This situation was reversed during the early stages of secondary cell wall synthesis (Berlin and Ramsey, 1971; Westafer and Brown, 1976); the ribosomes were almost exclusively in the polysome configuration, and free unattached ribosomes were rarely found.

Microtubules, oriented parallel to the fibrils in the cell wall, were observed in all stages of cotton fiber development. Particles, approximately 15-20 nm in diameter, were observed between the plasma membrane and secondary cell wall and were oriented in chains parallel with the microtubules (Figure 26). An intriguing possibility is that these particles may represent membrane-bound cellulose synthetase enzyme complexes (Brown and Montezinos, 1976) responsible for the synthesis of the secondary cell wall. Particles were observed between the

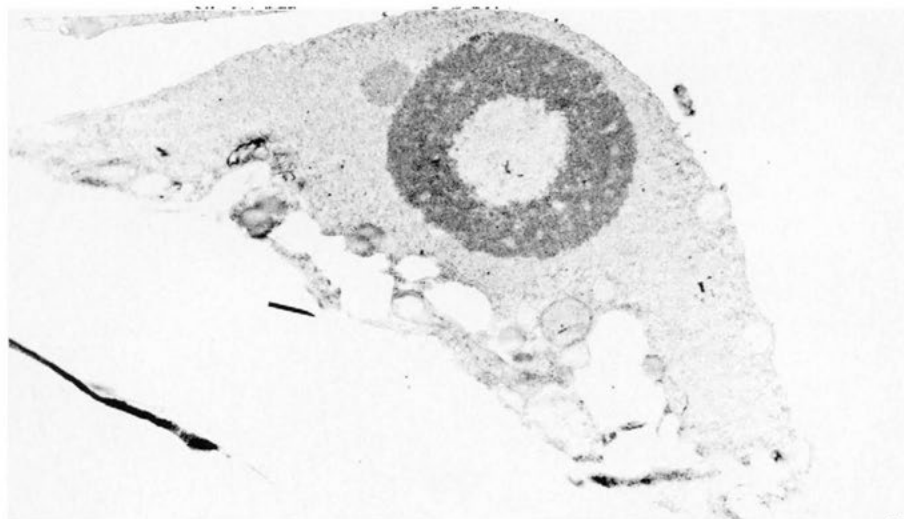


Figure 22. The nucleus of a 2-day postanthesis fiber contains an enlarged "bull's-eye" nucleolus. The nucleus is elliptically shaped and displaced to one side of the central vacuole. (7,000X magnification).



Figure 23. The nucleus of a 16-day postanthesis fiber is irregularly shaped and lacks heterochromatin and nucleoli. Several mitochondria with numerous cristae, plasma membrane-associated vesicles and the primary cell wall are shown. (37,500X magnification).

plasma membrane and primary cell wall as early as 2 days postanthesis; however, the frequency of particles observed increased tremendously during secondary wall synthesis.

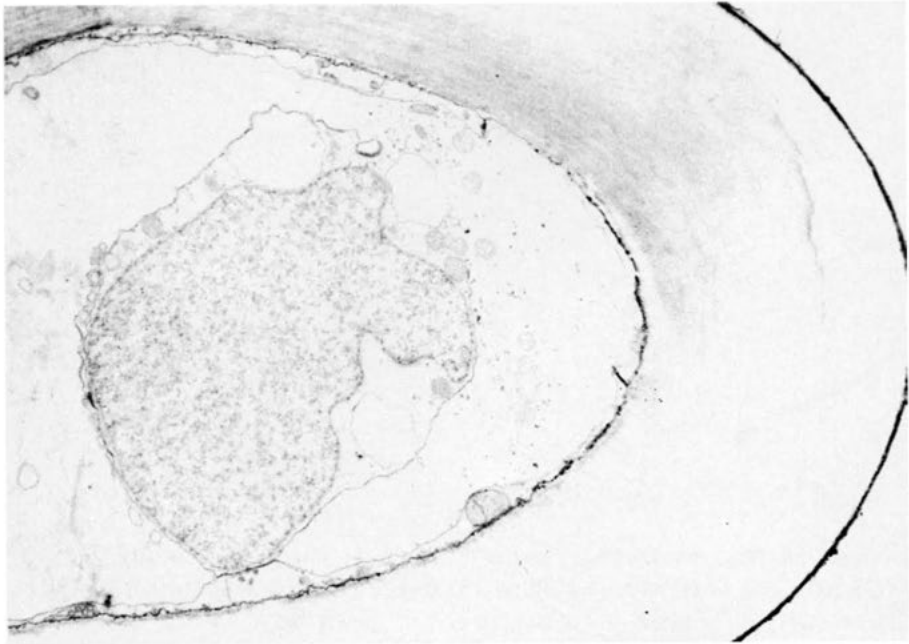


Figure 24. The nucleus of a 37-day postanthesis fiber is irregularly shaped, but structurally intact. Nuclei in old fibers lack a nucleolus and condensed chromatin. The mitochondrial cristae appear less numerous than in younger fibers. The characteristic secondary cell wall of a lint fiber is shown. (13,000X magnification).

Additional fibers were observed to initiate elongation at 6 days postanthesis (Figure 27). These late initiating fibers had dark cytoplasm containing many lipid bodies. The irregularly shaped nucleus was enlarged, had migrated from its central position in the epidermal cell toward the mid-region of the developing fiber and contained a large, elliptical nucleolus. Elongating dark and light cotton fibers could be distinguished at 7 days postanthesis (Figure 28). Farr (1933) observed that after the 6th day postanthesis, there was a marked contrast in fibers of different lengths. The longer ones, through increased vacuolation, became more and more hyaline and the shorter ones, in addition to their usual cytoplasmic density, became more opaque due to the apparent presence of a pigment in the cell cytoplasm. Epidermal cells of the seed coat accumulated mucilaginous and tannin-like compounds in the cell vacuoles a few days after anthesis (Lang, 1938). The presence of these substances in the vacuoles of the fuzz fibers was taken as evidence that such fibers originated several days after anthesis; fibers produced 1 to 2 days postanthesis did not contain these substances. Reeves (1936) and Brown and Ware (1958) also reported that in some varieties the lint and fuzz fibers were

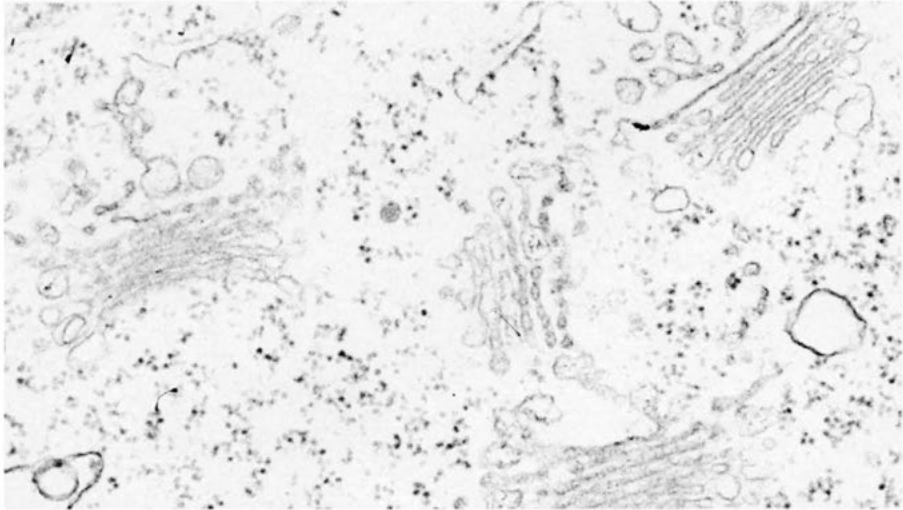


Figure 25. The cytoplasm at the tip of a 2-day postanthesis fiber. Numerous dictyosomes with cisternae containing fibrils similar to the fibrils found in the primary cell wall of the cotton fiber are shown. Most of the ribosomes are unattached, but many are in a helical configuration. (57,000X magnification). (From Ramsey and Berlin, 1976b).

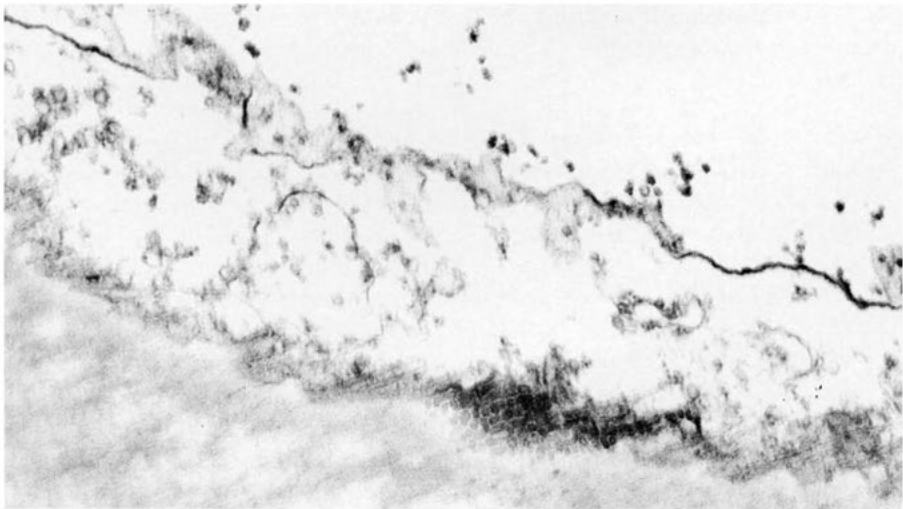


Figure 26. A tangential section of a 24-day postanthesis fiber. Particles located between the secondary cell wall and the plasma membrane apparently extend outward from microtubules in the cytoplasm. The possibility exists that the particles are membrane-embedded cellulose synthetases (Brown and Montezinos, 1976). (66,000X magnification).

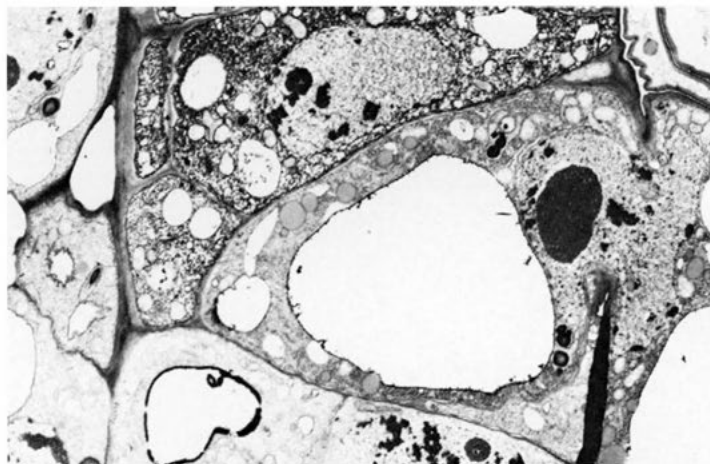


Figure 27. A slightly tangential section of the epidermal layer at 6 days postanthesis shows an initiating fuzz fiber. The fiber nucleus is enlarged, irregularly shaped and has migrated toward the fiber tip. An enlarged nucleolus is shown. The cytoplasm contains numerous vacuoles and lipid bodies. (8,500X magnification.)

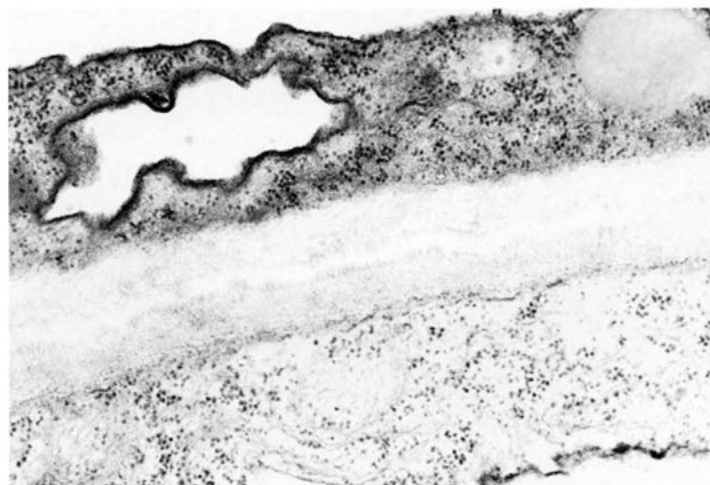


Figure 28. Portions of a light and dark fiber at 7 days postanthesis. The lint fiber has light cytoplasm and the fuzz fiber has an electron dense cytoplasm. Free, unattached ribosomes characteristic of the elongation stage are present in both fiber types. (38,000X magnification.)

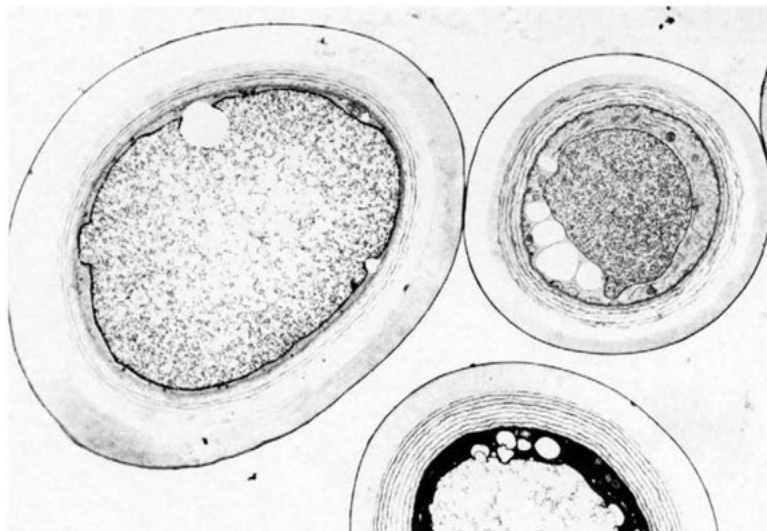


Figure 29. Cross-sections of fuzz fibers from Florida greenseed at 24 days postanthesis. Fuzz fibers are characterized by lamellae in the secondary cell wall, by dark cytoplasm and by pigments in the central vacuole. (6,300X magnification).

pigmented differently, and frequently pigment was present in the fuzz fibers when it was entirely absent from the lint fibers. The light and dark fibers were, therefore, identified as lint and fuzz fibers, respectively (Figure 28). Both types of fibers had free, unattached ribosomes (Figure 28) during the elongation stage of fiber development.

An electron microscopic examination of known fuzz fibers, e.g., the green fuzz from Florida greenseed (Figure 29), identified the dark fibers as fuzz fibers (Watson and Berlin, 1973). The fuzz fibers had dark cytoplasm similar to the cytoplasm of the initiating fiber shown at 4 days postanthesis. Other fibers had a light cytoplasm characteristic of lint fibers observed at anthesis and at one day postanthesis and, thus, can be identified as lint fibers (Figure 30). To repeat, fuzz fibers could be distinguished from lint fibers by their darker cytoplasm and greater amounts of condensed pigment in the lumen of the central vacuole. Additionally in older fibers, alternating light and dark rings (lamellae) were observed in the secondary cell wall of fuzz fibers (Figure 29). Lamellae, dark cytoplasm and large quantities of pigment were not observed in lint fibers (Figure 30). Cross sections of 24-day postanthesis fuzz fibers revealed that the number of lamellae was not the same in every fuzz fiber (Figure 29). Therefore, it is extremely doubtful that the fuzz fiber lamellae are equivalent to the temperature-related ring structures found in swollen cotton fibers by Grant *et al.* (1966). The density of the cytoplasm and the amount of pigment present in the central vacuole

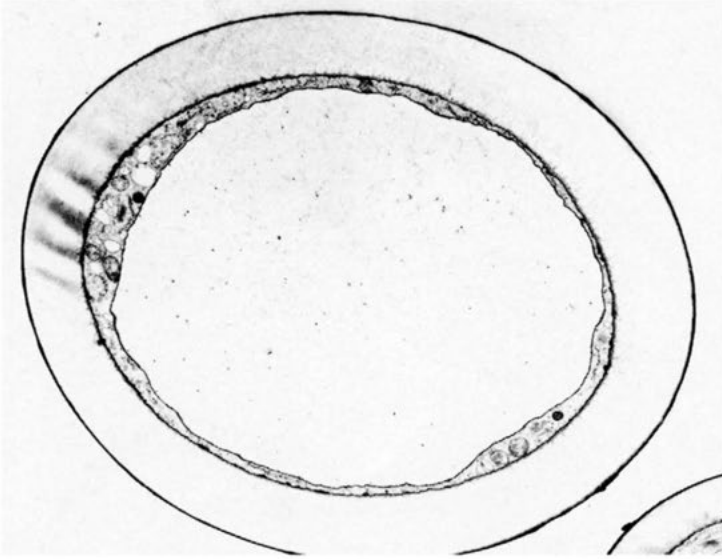


Figure 30. Cross-section of a lint fiber at 19 days postanthesis. Lamellae are not visible in the secondary cell wall, the cytoplasm is light and contains numerous organelles. Only a small amount of pigment is present in the central vacuole. (10,500X magnification).

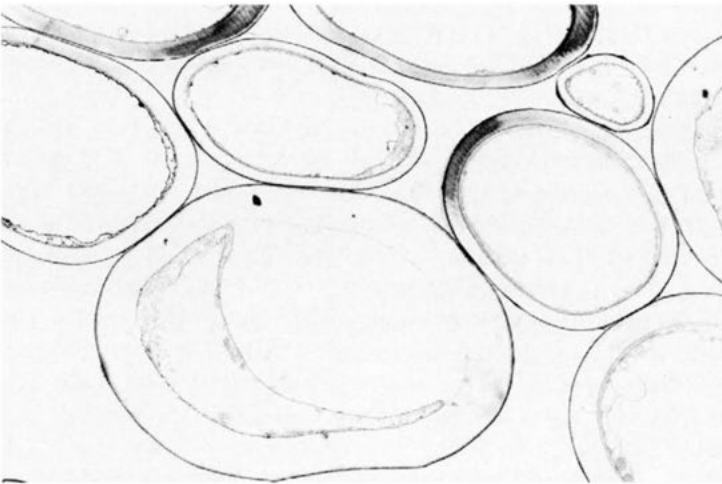


Figure 31. Cross-sections of lint fibers at 37 days postanthesis. Considerable variation in the thickness of the secondary cell wall is obvious. (6,000X magnification).



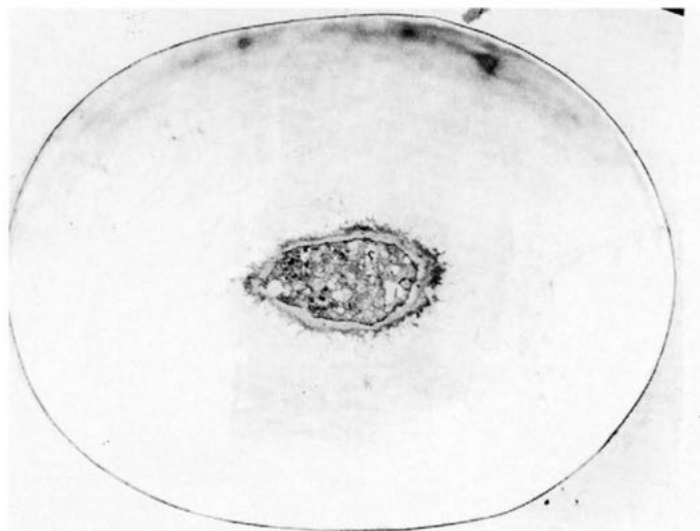


Figure 32. Cross-section of a lint fiber at 37 days postanthesis. An extremely thick secondary cell wall has reduced the lumen of the fiber. Such a thick cell wall is uncommon for lint fibers. (9,000X magnification).

varied considerably in fuzz fibers (Figure 29).

Cross sections of fibers at 37 days postanthesis revealed that some fibers at the chalazal end of the ovule had thin, immature, secondary cell walls (Figure 31). Other fibers were observed with thick secondary walls with the lumen of the fiber reduced to a small diameter due to cellulose deposition on the inner edge of the wall during formation of the secondary cell wall (Figure 32). The nonuniformity of cotton fiber cell walls has been quantitatively documented (Moore, 1941; Berlin *et al.*, 1981).

The ability to distinguish lint from fuzz fibers proved valuable in an examination of fiber bases. The base of the lint fiber has an exceedingly thin secondary cell wall (Figure 33). The paradermal expansion of the nonfiber epidermal cells squeezes the lint fiber base providing the elbow, shank and foot arrangement characteristic of the lint fiber (Fryxell, 1963). Most of the nonfiber epidermal cells synthesized a thick secondary cell wall with lamellations, and these cells were also filled with phenolic substances (Figure 34). The similarity between the nonfiber epidermal cells and the fuzz fibers with respect to these two features was striking.

The electron micrographs presented so far were obtained from cells that were not allowed to dry during specimen preparation. Obviously, when the boll opened under field conditions these cells did dry, shrink and distort. Upon drying, the shank of the lint fiber was crushed by the surrounding epidermal cells (Figure 35)

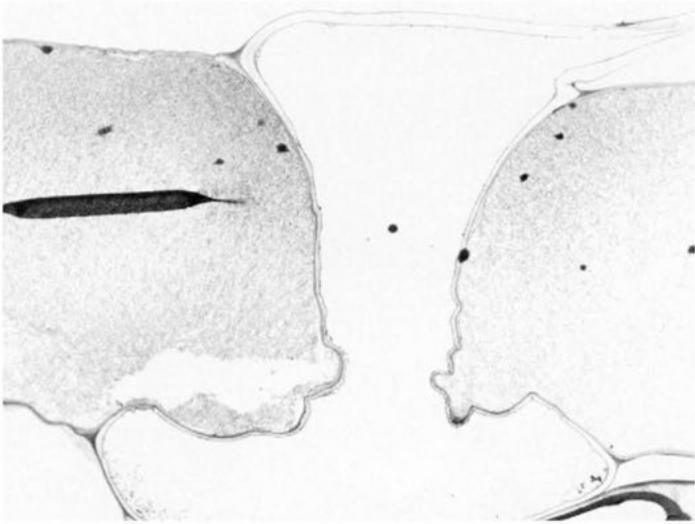


Figure 33. Median longitudinal section of the base of a lint fiber at 37 days postanthesis. A thin, immature secondary cell wall is found at the base of lint fibers. Lateral pressure from the adjacent, pigment-filled, epidermal cells has constricted the middle of the fiber base causing it to protude slightly below the bottom of the epidermal layer. (4,200X magnification).

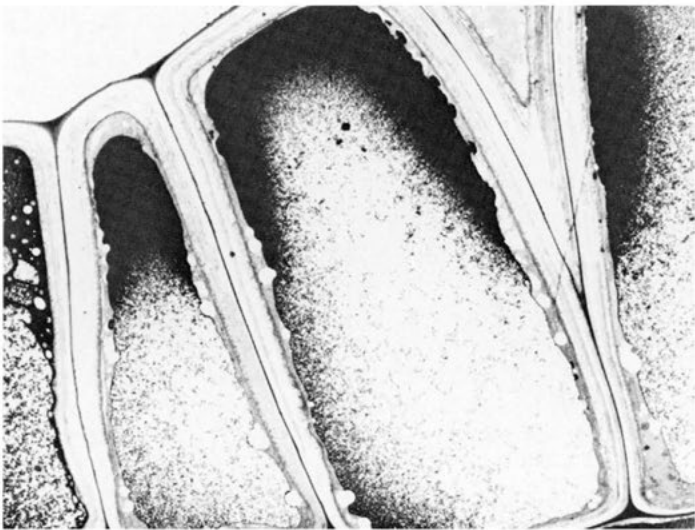


Figure 34. Median longitudinal section of the epidermal layer at 24 days postanthesis. The epidermal cells over most of the seed surface have thick secondary cell walls containing lamellae similar to that found in fuzz fibers. The epidermal cells also contain pigments in their central vacuoles. (3,500X magnification).



Figure 35. The base of a dried lint fiber from an opened boll sample. The thin secondary cell wall of the lint fiber base has been crushed by the adjacent cells that distort and shrink upon drying. (6,000X magnification).

and, because of the nearly nonexistent secondary cell wall in this region, the lint fibers lacked a firm connection to the seed. The absence of the thick secondary cell wall at the base of the lint fiber is of obvious importance to the ginning process. In contrast, the base of the fuzz fiber had a thick secondary cell wall that extended into the epidermal layer (Figure 36). Upon drying and subsequent crushing by adjacent epidermal cells, the thick secondary cell wall at the base of the fuzz fibers literally anchored the fuzz fiber to the seed. The effect of drying on the bases of lint and fuzz fibers is diagrammed in Figure 37.

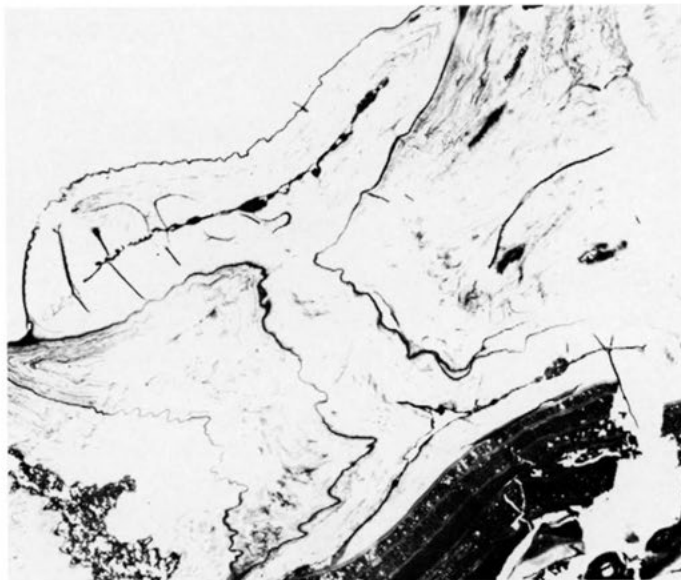


Figure 36. The base of a dried fuzz fiber from an opened boll sample. The thick secondary cell wall of the fuzz fiber extends into the epidermal layer and, presumably, is the major contributing factor in fuzz fibers remaining on seeds after ginning. (3,500X magnification).

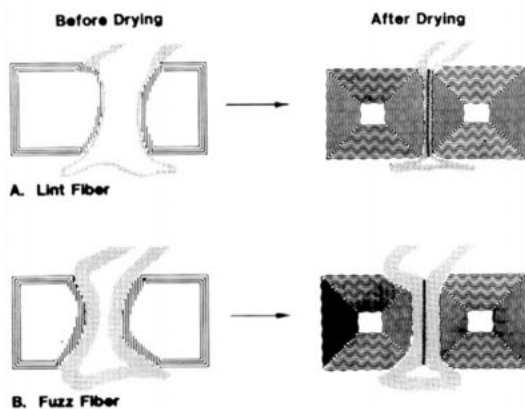


Figure 37. Diagram of the effects of drying on lint and fuzz fiber bases. Lint fibers have exceedingly thin secondary cell walls at their bases, whereas fuzz fibers have thick secondary cell walls that extend into the epidermal layers. The shank region of the lint fiber is crushed by adjacent epidermal cells upon drying resulting in a tenuous attachment of the lint fiber to the seed. The dried fuzz fiber is essentially anchored into the epidermal layer by virtue of the thick secondary cell wall at its base.

## AUTORADIOGRAPHIC ANALYSES OF THE EPIDERMAL LAYER

Fiber primordial differentiation and fiber development are exceedingly complex events. The morphology of these stages suggested underlying changes in subcellular functions. We have followed the incorporation of various radioactive precursors into these cells using autoradiography to identify some of these physiological changes.

The ability to grow cotton ovules in culture (Beasley, 1973) facilitated the study of fiber development without experimental complications from the ovary or other plant parts. In our autoradiographic studies, radioisotopes were added to Beasley's culture media, and their cellular incorporation was correlated with ovule and fiber development. Specifically, DNA synthesis, RNA synthesis, protein synthesis and an indication of possible phenolic synthesis were examined using tritiated thymidine, uridine, amino acids (a batch mixture of glutamic acid, glycine, leucine and lysine) and phenylalanine, respectively. The autoradiographic techniques, including the use of Nomarski contrast to examine unstained sections, was previously described (Smutzer and Berlin, 1976). The plant material used for the autoradiographic studies was the commercial cultivar DPL 16.

### THYMIDINE

The uptake of tritiated thymidine was expressed as numerous dots (exposed silver grains) in autoradiographs (Figure 38). Approximately 10 percent of the epidermal cells incorporated tritiated thymidine after a 15 minute incubation at 12 days preanthesis (Figure 39). A linear decrease in thymidine uptake was observed until 2 days preanthesis when none of the cells were labeled after a 15 minute incubation (a prolonged incubation of 12 hours revealed less than 5 percent of these epidermal cells had incorporated thymidine). After this absence of labeling, there was an increase in thymidine incorporation until 1 day postanthesis when 7 percent of the epidermal cells were labeled with a 15 minute incubation (Figure 39). Thereafter, a sharp drop in uptake occurred and very few epidermal cells were labeled between 3 and 6 days postanthesis. Thymidine was not incorporated into the epidermal layer after 6 days postanthesis (even with 12-hour incubation times).

The arrest of cell division in the epidermis at 2 days preanthesis was concomitant with differentiating fiber primordial cells (Aiyangar, 1951; Ramsey and Berlin, 1976a,b). These observations suggested that cell division and cell differentiation were antagonistic processes in the cotton epidermal cells. The change from synthesis of substances for the cell's own maintenance to synthesis of their differentiated products is often accompanied by the slowing or cessation of cell division (Elbert and Kaighn, 1966). Incorporation of thymidine by nonelongating cells was observed at anthesis; therefore, the early stages of cell elongation in neighboring fibers had little, if any, effect on the mitotic activity of nonelongating



Figure 38. Autoradiograph of the epidermal layer at 2 days postanthesis showing tritiated thymidine incorporation. The uptake of thymidine is shown by the dots overlying the cells. The nuclei of two cells in the epidermal layer are heavily labeled. Cytoplasmic labeling results from the incorporation of thymidine into mitochondria and proplastids. Nomarski interference contrast light micrograph of unstained section. (625X magnification).

epidermal cells. The cell cycle was not synchronized as both nonlabeled and labeled cells were present at the same time.

The epidermal cells did not incorporate thymidine after 6 days postanthesis. Ultrastructural observations suggested that the vacuoles of these cells enlarged and became filled with phenolic materials, and the nuclei were pushed to the sides of these cells. These chemical and morphological changes were probably related to the cessation of DNA synthesis in the epidermis, and it appeared unlikely that these differentiated cells would undergo mitosis after 8 days postanthesis. Farr (1931, 1933), however, found evidence of micropylar cells in apparent late telophase as late as 12 days postanthesis.

Hence, as a whole, the tritiated thymidine studies showed that ovule enlargement was due primarily to cell division up to 3 days postanthesis; whereas after this time, ovule enlargement (discussed later) was due to an increase in the size of

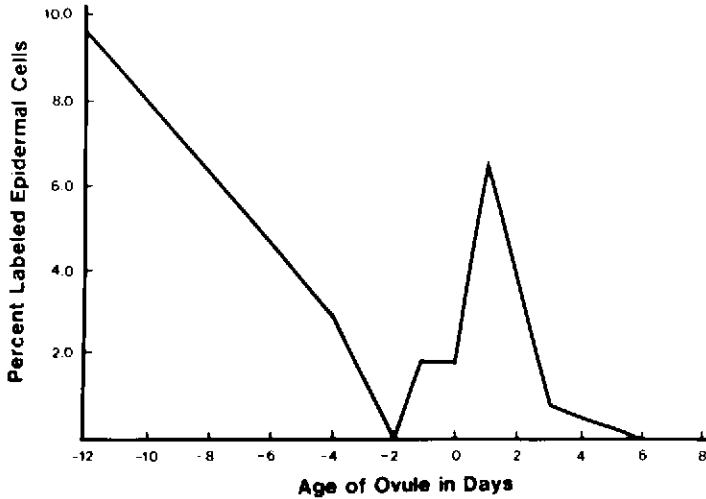


Figure 39. The percent labeled epidermal cells at different ovule ages after a 15-minute incubation in tritiated thymidine. Thymidine was not incorporated into the epidermal layer after 6 days postanthesis.

existing cells.

The assumption was made that cells synthesizing DNA were preparing to enter mitosis. Whether or not cell division occurred after thymidine incorporation could not be determined. Although considered unlikely, it is possible that the thymidine may have been degraded to an undetermined labeled fraction. Incorporation of such fractions into the nonnuclear portions of labeled cells appeared to be minimal, and it is likely that cytoplasmic labeling may have represented mitochondrial and proplastid incorporation of thymidine. A second alternative was that labeled thymidine may have been incorporated into DNA undergoing gene amplification in differentiating fiber primordial cells. This explanation appears unlikely since the percentage of cells incorporating thymidine in the epidermal layer during differentiation into fibers was low. If fiber primordial cells were undergoing gene amplification, at least 10 percent of the epidermal cells would have to be labeled since approximately 1 in 10 epidermal cells initiate elongation at anthesis. No incorporation was observed over nuclei in elongating fibers after anthesis. These observations suggested that gene amplification did not occur during fiber development at any time tested. In summary, it appears that the best interpretation of these experiments is that thymidine incorporation occurred as DNA was synthesized in preparation for cell division.

## URIDINE

The incorporation of tritiated uridine by fiber primordia and elongating fibers

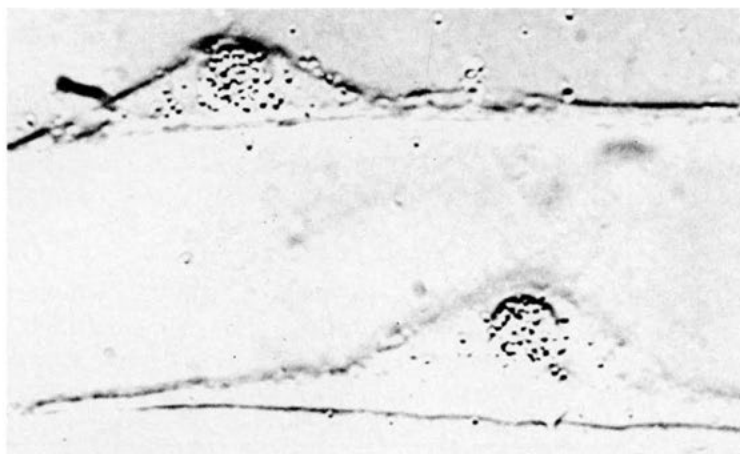


Figure 40. Autoradiograph of tritiated uridine incorporation in fibers at 2 days postanthesis. The bulk of the exposed silver grains over the large nucleoli in the developing fibers is suggestive of mRNA synthesis. Nomarski interference contrast micrograph of an unstained section. (1450X magnification). (From Smutzer and Berlin, 1976).

occurred from 1 to 6 days postanthesis (Figure 40). The incorporation at 6 days postanthesis was slight and virtually no uridine incorporation occurred in fibers after 6 days postanthesis. These results indicated that the bulk of fiber RNA was made from 1 to approximately 5 days postanthesis. The greatest uptake of uridine in fiber cells occurred at 3 days postanthesis when both total cellular and nucleolar incorporation were highest. The increase in nucleolar incorporation occurred in the enlarged nucleolus previously described in the morphological studies.

Most, if not all, of the cellular RNA occurring in fiber cells was synthesized prior to 6 days postanthesis. Differing chemical compositions of primary and secondary cell walls (Frey-Wyssling and Muhlethaler, 1965) indicate the probability of different enzymes functioning during the elongation and thickening stages. Indeed, biochemical evidence suggests that different enzyme systems are necessary for primary and secondary cell wall synthesis in cotton (Delmer *et al.*, 1974). The synthesis of fiber RNA prior to 6 days postanthesis suggested the existence of a long-lived mRNA that would be translated into the enzymes required for secondary cell wall synthesis sometime after 16 days postanthesis. Clay *et al.* (1975) and Walters and Dure (1966) have found evidence of long-lived messenger RNA in germinating cotton seeds, and the extension of its presence in fiber development seems logical. Since it was not experimentally feasible to observe every time period in fiber development, small spurts of RNA synthesis could have been missed in this study, but it seems apparent that all or nearly all of the RNA utilized in fiber development was synthesized shortly after anthesis.



In these studies, two assumptions were made in comparing uridine incorporation to actual RNA synthesis. First, it was assumed that the cellular uridine pool did not change with time. If this is not the case, an increase in the uridine pool would necessitate an increase in the amount of tritiated uridine to give the same level of labeling. Secondly, it was assumed that the ability of uridine to enter fiber cells did not change during the different stages of fiber development. These changes could not be detected by the methods employed in our study.

### AMINO ACIDS

The ability of the epidermal layer to synthesize proteins was assayed by their uptake of a mixture of four radioactive amino acids (glutamic acid, glycine, leucine and lysine). The uptake of amino acids in the epidermal layer occurred from the earliest day tested (6 days preanthesis) until 3 days postanthesis with resumption of uptake at 22 days postanthesis. No uptake was observed at 6, 16, or 33 days postanthesis. Peak incorporation occurred at 4 days preanthesis, anthesis and to a lesser extent at 3 days postanthesis. Interestingly, the subepidermal cells incorporated a greater amount of the mixture of radioactive amino acids than did the epidermal cells at times near anthesis (Figure 41).

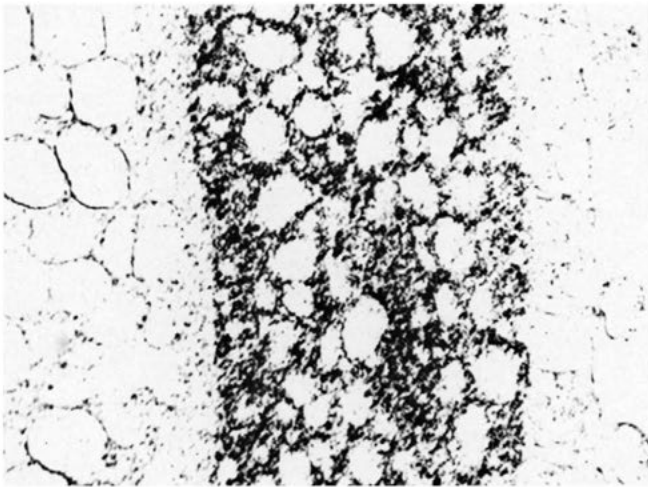


Figure 41. Autoradiograph of 1 day postanthesis cotton ovule exposed to a mixture of amino acids (glutamic acid, glycine, leucine and lysine). The young, elongating fibers are outlined by cytoplasmic incorporation of the amino acids. The majority of the label occurs in the subepidermal cells. Nomarski interference contrast micrograph of an unstained section. (625X magnification).

Several ultrastructural changes occurred in epidermal cells that may have been related to increased uptake of amino acids from 4 days preanthesis to anthesis

(Berlin and Ramsey, 1970; Ramsey and Berlin, 1976a,b). First, there was an increase in the cytoplasm to cell size ratio of epidermal cells prior to anthesis. Second, shortly before anthesis, the previously electron-light vacuoles contained dense phenolic pigments. Third, an increase in the endoplasmic reticulum and in the number of free ribosomes was found in fiber primordia prior to their initiation of elongation. Fourth, increased numbers of dictyosomes were found in epidermal cells that had initiated elongation. All of these morphological changes may have been related to increased amino acid incorporation into cellular proteins. Metabolism of proteins are necessary for an increase in cytoplasmic ground substance, ribosome production and dictyosomal synthesis. A multitude of new enzymes may be necessary to direct the preanthesis synthesis of electron dense phenolic pigments.

From fiber initiation at anthesis to 3 days postanthesis, rapid ultrastructural changes may have again been related to increased amino acid incorporation found at these stages of ovule development. The elongation of certain epidermal cells from 10  $\mu\text{m}$  to over 2,000  $\mu\text{m}$  in length, with most of the cell volume taken up by a large central vacuole, dictated a large increase in membrane expansion. Geometric calculations of elongated cells indicate over a 2,000 fold increase in the surface area of the plasma membrane and tonoplast had occurred by 3 days postanthesis. The increase in membrane expansion may have been related to some or all of the increased amino acid incorporation occurring from 4 days preanthesis to 3 days postanthesis.

During the period of primary cell wall synthesis, the bulk of amino acid label in the fiber was observed prior to 6 days postanthesis. With fiber length continuing until approximately 27 days postanthesis (Schubert *et al.*, 1973) and ultimately increasing 3,000 times in length (Watson and Berlin, 1973), apparently most of the proteins were synthesized prior to 6 days postanthesis. Wakelyn (1975) found evidence of free amino acids occurring during primary wall extension. Free amino acids would have been removed by our method of specimen preparation and would not have been detected by autoradiography. It is possible that after 6 days postanthesis, protein components for continued membrane and cell wall extension were synthesized from a free amino acid pool. Another alternative is that by 6 days postanthesis until 22 days postanthesis, the labeled amino acids may have been incapable of entering the fibers and, if such conditions existed, would not have been detected by autoradiography at these times. The resumption of incorporation after 22 days postanthesis would tend to negate this latter possibility.

### **PHENYLALANINE**

The epidermal cells and the young fibers were extensively labeled with phenylalanine at 1 day postanthesis (Figure 42). The cytoplasm of the subepidermal cells was also labeled with this amino acid, and the amount of subepidermal labeling increased in older cottonseeds.

The decamination of phenylalanine by phenylalanine ammonia lyase is an early step in the formation of numerous phenolic compounds (Walker, 1975; Ribereau-

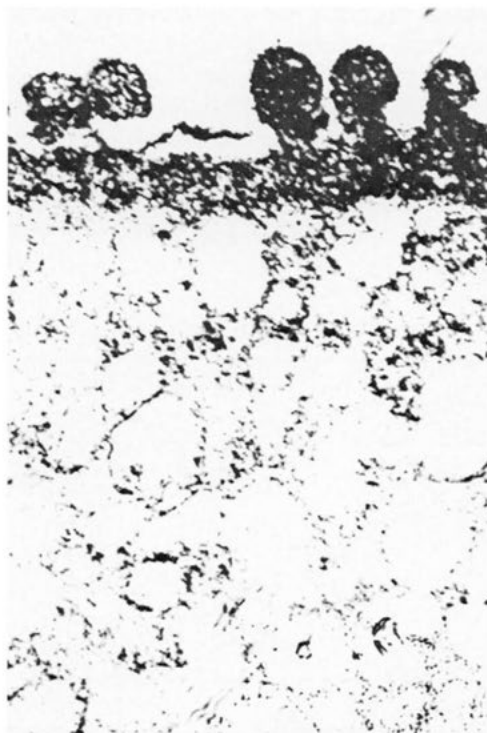


Figure 42. Autoradiograph of 1 day postanthesis ovule incubated in tritiated phenylalanine. The cells in the epidermal layer as well as the young fibers are extensively labeled, probably due to the incorporation of phenylalanine into phenolics. Nomarski interference contrast micrograph of an unstained section. (625X magnification).

Gayon, 1972). We hoped to get some indication of phenolic biosynthesis in the epidermal layer by comparing phenylalanine incorporation to the mixture of amino acids. As an amino acid, phenylalanine incorporation would be expected to closely parallel the incorporation of other amino acids. Any variation in the uptake of phenylalanine from the other amino acids would be presumptive evidence for phenolic biosynthesis, especially if the activity could be positively correlated with the cytochemical localization of phenolics.

The difference in autoradiographic results between phenylalanine and the four amino acids used to detect protein synthesis, especially at 1-day postanthesis (compare Figures 41 and 42), suggested phenylalanine might be incorporated into phenolics (especially in the epidermal layer and young fibers), as well as into proteins. Phenylalanine uptake in the epidermal layer closely paralleled the phenolic activity observed in these cells in our morphological studies.

The combining of phenolics with proteins may regulate cell development by altering enzymes and organelles (Swain, 1965). Naturally occurring phenolics may act *in vivo* as inhibitors or activators of indole acetic acid (IAA) by interacting with IAA oxidase (Henderson and Nitch, 1962; Reynolds and Maravolo, 1973; Zenk and Muller, 1963). Kosmidou-Dimitropoulou reported that IAA stimulated fiber elongation (Chapters 23 and 25).

A heavy labeling of subepidermal cells with phenylalanine at later stages of ovule development was probably related to the increased tannin content in the integuments of the ovule.

Table 1. Comparison of cotton ovules at anthesis.

Kultivar or strain	Surface area (mm <sup>2</sup> )	Number of surface cells	Uncorrected fibers/seed	Surface with fibers (%)	Corrected fibers/seed
Florida Greenseed	4.40	54,000	16,000	80	12,800
CB 3051	3.71	55,200	11,300	50	5,700
Dunn 56C	4.06	61,700	10,800	60	6,480
Sea Island	3.97	70,800	1,000	30	300
Texas 429	4.03	69,400	0	0	0
Isogenic Naked	3.96	66,200	0	0	0
Mexican Acala	3.40	57,500	0	0	0

## THE SEED SURFACE

The number of epidermal cells and fibers per seed was obtained by calculating the surface area of the cottonseed (Figure 43) assuming it to be an eccentric spheroid (Farr, 1931) and, by proportion, converting the number of features in the area of a scanning electron micrograph of the seed surface (a magnification of about 500X was required) to the number of cells and/or fibers per surface area of the cottonseed.

Examination of ovule surfaces on the day of anthesis with the scanning electron microscope (Figure 44) revealed about 60,000 cells per ovule regardless of the cotton type (Table 1). However, cotton types differed greatly in the pattern of fiber initiation. For example, naked seed strains showed no fiber elongation at anthesis, whereas Dunn 56C and Florida Greenseed had 50 and 80 percent, respectively, of their surfaces covered with fibers at anthesis (Table 1). The corrected number of fibers per seed was obtained by counting the number of fibers in the area containing fibers and multiplying by the percent area of the seed with fibers.

The cell count data go hand-in-hand with our autoradiographic results of thymidine incorporation, i.e., cell division in the epidermal layer ceases at about 6 to 8 days postanthesis. Subsequent seed enlargement (Figure 43) was accompanied by increased cell size and not increased cell number.

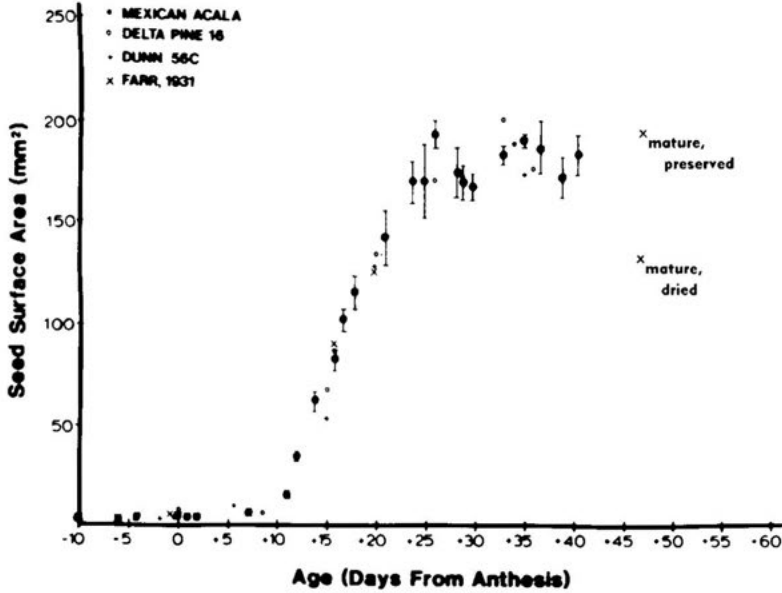


Figure 43. The increase in cottonseed surface area with age. The surface area was calculated assuming the cottonseed to be an eccentric spheroid (Farr, 1931). Farr's data for 5 cottonseeds are included and illustrate the shrinkage of the cottonseed caused by aging. The vertical bars indicate 2 standard errors of the mean.

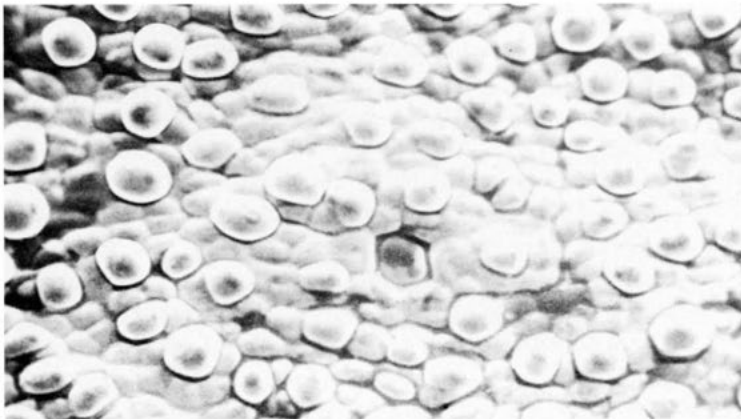


Figure 44. Scanning electron micrograph of the cottonseed surface at 1 day post-anthesis showing fiber primordia initiating elongation. (500X magnification). (From Ramsey and Berlin, 1976a).

Our previous results with the transmission electron microscope afforded us the ability to distinguish lint and fuzz fibers with the scanning electron microscope. Very simply, lint fibers have thin-walled bases and fuzz fibers have thick-walled bases. It was necessary to hydrolyze the fibers with concentrated sulfuric acid to observe the surface of fiber-bearing seeds (Figure 45). The lint and fuzz fibers

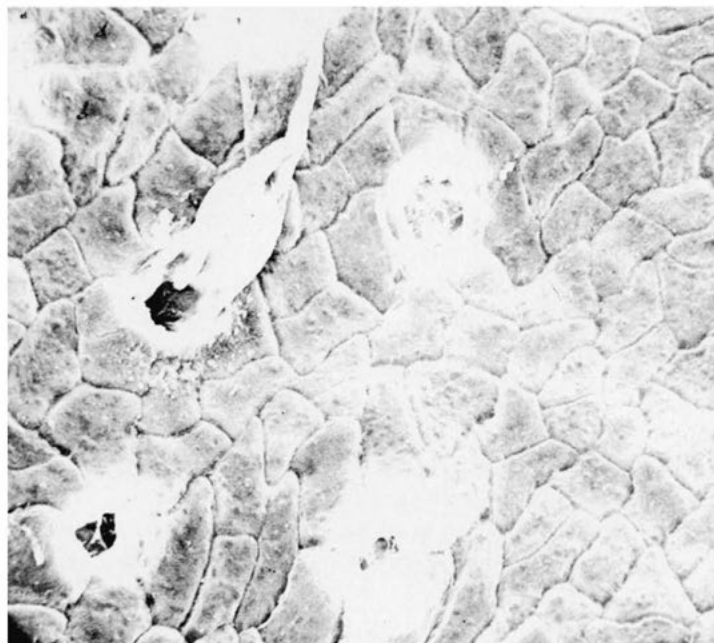


Figure 45. Scanning electron micrograph of the seed surface of a mature, but never-dried seed. Most of the fibers were hydrolyzed with a short rinse in concentrated sulfuric acid to expose the seed surface. The outlines of the epidermal cells are obvious. A single fiber is shown emerging from the epidermal layer. The thick-wall surrounding the hole in the epidermal layer in the middle of the micrograph represents the base of a fuzz fiber. (800X magnification.).

could be distinguished on the basis of their secondary cell wall. Comparing cell and fiber counts from about ten strains and varieties, we found the lint fiber to epidermal cell ratio varied tremendously between different cotton types. It appeared that for most naked seed strains there was one lint fiber for every 21 epidermal cells. What would happen if this ratio could be altered from 1:21 to 2:21? In fact, this happened with commercial cultivars. For example, at 15 days postanthesis the cultivar Dunn 56C had about 31,000 lint fibers and 340,000 epidermal cells, or a lint fiber to epidermal cell ratio of 1:11. These numbers appear to be genetically determined, and it is possible that this represents a genetic trait that might be

beneficially incorporated into commercial cultivars. Would a change in this ratio from 1:11 to 1:10 be reflected by a 10 percent increase in yield? The fuzz fiber to epidermal cell ratio on Dunn 56C was about 1:13. It is interesting that the presence of fuzz fibers on cottonseeds is positively correlated with lint fiber yield.

## SUMMARY

In summary, the epidermal cells showed a progressive increase in size from 23 days preanthesis to about 20 days postanthesis. Mitotic figures were found in the youngest samples examined through six days postanthesis. Similarly, thymidine incorporation occurred in the epidermal layer (except at 2 days preanthesis) until cessation at six days postanthesis. The number of epidermal cells increased from about 1,000 at 23 days preanthesis to about 60,000 at anthesis and to nearly 350,000 cells at 6 days postanthesis. If the cell divisions were synchronous and all cells participated, these numbers would mean that there were 6 cell divisions from 23 days preanthesis to anthesis and 3 cell divisions from anthesis to 6 days postanthesis. The time required for a cell division would decrease from 5 days to 2 days for the pre- and postanthesis periods, respectively. The surface area of the epidermal cells increased from approximately  $60 \mu\text{m}^2$  at anthesis to about  $200 \mu\text{m}^2$  at 15 days postanthesis. The major increase in cottonseed surface area (in most cottons from about 10 to  $200 \mu\text{m}^2$ , respectively) occurred between 10 and 25 days postanthesis.

The accumulation of phenolic compounds in vacuoles just prior to anthesis and the preferential incorporation of phenylalanine into the epidermal layer at anthesis suggests a prominent role for these compounds in epidermal cells. Indeed, phenolic compounds appeared to play a role in the differentiation of fiber primordia. The epidermal cell vacuoles became filled with phenolics, and some of these compounds were subsequently dispersed into the cytoplasm of fiber primordia. On the other hand, the phenolics in the nonfiber cells remained within the vacuoles. It is possible that these observations are a morphological manifestation of a mechanism regulating fiber differentiation. Phenols can regulate IAA activity by interacting with IAA oxidase, the enzyme that destroys IAA (Henderson and Nitch, 1962). It would appear that the cells in the epidermal layer are subject to individual growth regulation via phenolic compounds. A working hypothesis would be that this phenomena would regulate the fiber to nonfiber ratio and, possibly, the lint to fuzz fiber ratio on the cottonseed. This hypothesis should be amenable to testing using ovule culturing techniques (Beasley, 1973) to screen various phenolic substances.

The huge increase in phenolics in the epidermal cells at 8-10 days postanthesis is likely a reflection of a metabolic storage system that ultimately provides a protection device for the cottonseed (phenolics are known to confer disease resistance to plants, Ribreau-Gayon, 1972). (See also Chapter 38).

The morphological and autoradiographic observations suggested that RNA and protein synthesis may be stage specific and may occur well in advance of their

apparent need in the developing fiber. In particular, rRNA and mRNA required for secondary cell wall synthesis during the thickening stage appeared to be synthesized during the first 6 days postanthesis.

### ACKNOWLEDGMENTS

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