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RED-LIGHT-INDUCED ENDOSPERM PREPARATION FOR RADICLE PROTRUSION OF LETTUCE EMBRYOS

G. PSARAS, K. GEORGHIOU, AND K. MITRAKOS

Institute of General Botany, University of Athens, Athens 621, Greece

Light microscopic examination of red-light-treated *Lactuca sativa* achenes at various stages of imbibition revealed that, prior to visible germination (radicle protrusion), the endosperm cells of the micropylar end undergo rapid characteristic structural changes. The cytoplasm becomes highly vacuolated, and reserve materials are mobilized. Generally, these cells swell, while the cell walls remain PAS positive. In dark-, far-red, and red + far-red-light-treated achenes, however, the endosperm remains unchanged. The structural changes may be a prerequisite for endosperm rupture and radicle protrusion.

Introduction

The lettuce embryo is surrounded by a two-cell-layered endosperm with thick-walled cells full of storage materials (Borthwick and Robbins 1928). Biochemical work on the lettuce endosperm during germination indicates that several changes start about the time of radicle protrusion: the appearance of enzymes and their function (Halmer, Bewley, and Thorpe 1975, 1976), the mobilization of storage reserves (Leung, Reid, and Bewley 1979) and cell wall materials (Halmer, Bewley, and Thorpe 1978; Halmer and Bewley 1979), and a decrease in endosperm weight (Park and Chen 1974).

Jones (1974) reported extensive digestion of the walls of the endosperm cells at 15 h from the onset of imbibition but did not refer to any specific area of the endosperm. The exact stage of germination at which the digestion occurs is unknown. Pavlista and Valdovinos (1978), studying surface changes of the lettuce endosperm during germination by scanning electron microscopy, reported some cracks and pits on the micropylar end of the tissue and also reported that endosperm rupture occurs at these points of weakening after 10–12 h from the start of imbibition.

Since the experiments of Borthwick and Robbins (1928) on the influence of the endosperm on germination of lettuce achenes, much has been done to determine the exact role of this tissue in restricting radicle protrusion (Evenari and Neumann 1952; Ikuma and Thimann 1963; Scheibe and Lang 1965; Pavlista and Haber 1970; Nabors and Lang 1971). Mechanical restriction, intervention in the uptake of oxygen, secretion of chemical inhibitors (Evenari 1965), or a role as an osmotically active membrane (Speer 1974; Speer and Hsiao 1976) have been proposed as functions of the endosperm during embryo elongation.

We describe here certain structural changes in the endosperm cells at the micropylar end occurring just before radicle protrusion. These observations may help us understand the role of the endosperm and the mechanism of radicle protrusion.

Material and methods

Plant material and germination.—Lettuce achenes (*Lactuca sativa* L. 'Grand Rapids'; Carolina Biological Supply Co., Burlington, N.C.; 1978 harvest) were imbibed in darkness on two sheets of Whatman no. 1 filter paper in petri dishes at 25 ± 1 C in 3 ml of deionized water. Each dish contained 50 achenes. The dishes were separated into four groups: (1) kept in darkness (D), (2) incubated in darkness for 1 h and then exposed to red (R) for 1 min or (3) to far-red (FR) for 2 min or (4) to red followed by far-red (R + FR). After light treatment the achenes were returned to darkness.

Red-light illumination (R group) results in 96% ± 1% total germination after 24 h from the start of imbibition. In the other groups total germination percentage is nearly 0% (D, 4 ± 1; FR, 2 ± 1; R + FR, 2 ± 1). The time course of germination after red-light treatment shows that germination starts at 12 h from the onset of imbibition (fig. A).

Red light (625–700 nm, emission maximum at 660 nm, 3 W m^{-2}) was obtained from eight red fluorescent tubes (Philips TL 20 W/15) filtered through a red Plexiglas filter (Röhm, 501). Far-red light (700–800 nm, emission maximum at 750 nm, 5 W m^{-2}) was obtained from 10 white incandescent tubes (Philips philinea 6276X, 60 W) filtered through one red and two blue Plexiglas filters (Röhm, 501 and 627, respectively) 3 mm thick and a 5-cm water bath. All manipulations were conducted under a green safelight (525–575 nm, emission at 550 nm, 10 mW m^{-2}) obtained from a green fluorescent tube (F 15 T8. G. 6, 15 W Green Photo, General Electric) filtered through a red orange and a green filter (Röhm, 478 and 700, respectively) 3 mm thick.

Microscopy.—Achenes were fixed in 6% glutaraldehyde for 3 h at 4 C in 0.025 M phosphate buffer at pH 7, after 1, 6, 9, 12, and 15 h from the start of imbibition. After washing with buffer, the seed coat was removed by dissecting pins under a stereoscope,

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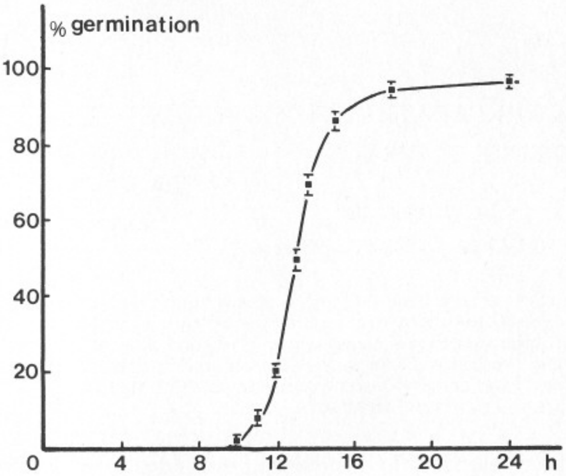


FIG. A.—Time course of germination of red-light-treated lettuce achenes.

and the embryos surrounded by the endosperm and parts of the integuments were postfixed in 1% osmium tetroxide in phosphate buffer for 3 h at 4 C. After dehydration in an ethanol series, followed by 30 min in propylene oxide, the tissue was embedded in Durcupan ACM (Fluka). The plant material was sectioned on an LKB pyramitome, placed on glass slides, and stained with toluidine blue (overall monochromatic staining obtained by employing 1% toluidine blue O made with 1% borax solution). For histochemical identification of carbohydrates on semithin sections (1–3 μm), the periodic acid–Schiff (PAS) reaction was performed (NEVALAINEN, LAITIO, and LINDGREN 1972).

Observations

Light microscopic examination of median longitudinal sections of lettuce achenes reveals a two-layered endosperm with thick-walled cells (figs. 1, 9, 14).

Achenes imbibed in darkness for 1 h have narrow lateral endosperm cells in a regular arrangement with thick cell walls (figs. 1, 3). The outer cell walls are obviously thicker than the inner ones (fig. 3). Adjacent cells in the micropylar area overlap. All endosperm cells have several wall protrusions and dense cytoplasm (figs. 1–3) with a poor vacuolar system and are rich in storage material. Lipid material (BORTHWICK and ROBBINS 1928) is abundant in micropylar and lateral endosperm cells (figs. 2, 3).

Achenes imbibed in darkness and achenes illuminated with FR or R + FR light (nongerminating achenes) show no change in endosperm cell structure at 6, 9, 12, and 15 h from the start of imbibition and also show the same characteristics as those imbibed for 1 h in darkness (cf. figs. 1–3 with figs. 4, 5 and 7, 8). Sections of the same achenes from 1, 6, 9, 12, and 15 h D, FR, and R + FR treated samples stained with PAS reagent show that the walls of all lateral and micropylar endosperm cells are strongly PAS positive (fig. 6).

Median longitudinal sections of R-treated achenes (germinating achenes) imbibed for 12 h reveal that the lateral and cotyledonary end cells of the endosperm (figs. 11, 16) have the same structure as those in achenes imbibed in darkness (fig. 5). In contrast, cells in the area facing the radicle tip have a completely different structure; they are highly vacuolated with clearly mobilized storage materials (figs. 12, 13) and, in general, seem to swell (figs. 10, 14, 15). The cell walls of the entire endosperm, including micropylar cells, are PAS positive (figs. 9–11, 14–16).

In endosperm cells of achenes with ruptured endosperms (12 or 15 h), the micropylar cells are obviously highly vacuolated, do not contain storage materials, and are spherical (figs. 15, 17–19). The structure of the lateral endosperm cells seems to remain unchanged (cf. fig. 20 with figs. 3, 5). Also, the walls of the endosperm cells are PAS positive for at least a short period after radicle protrusion (cf. figs. 17–20 with 12–13 and figs. 14–16 with 9–11).

Structural changes in endosperm cells are relatively rapid prior to germination and are influenced by phytochrome activation (table 1).

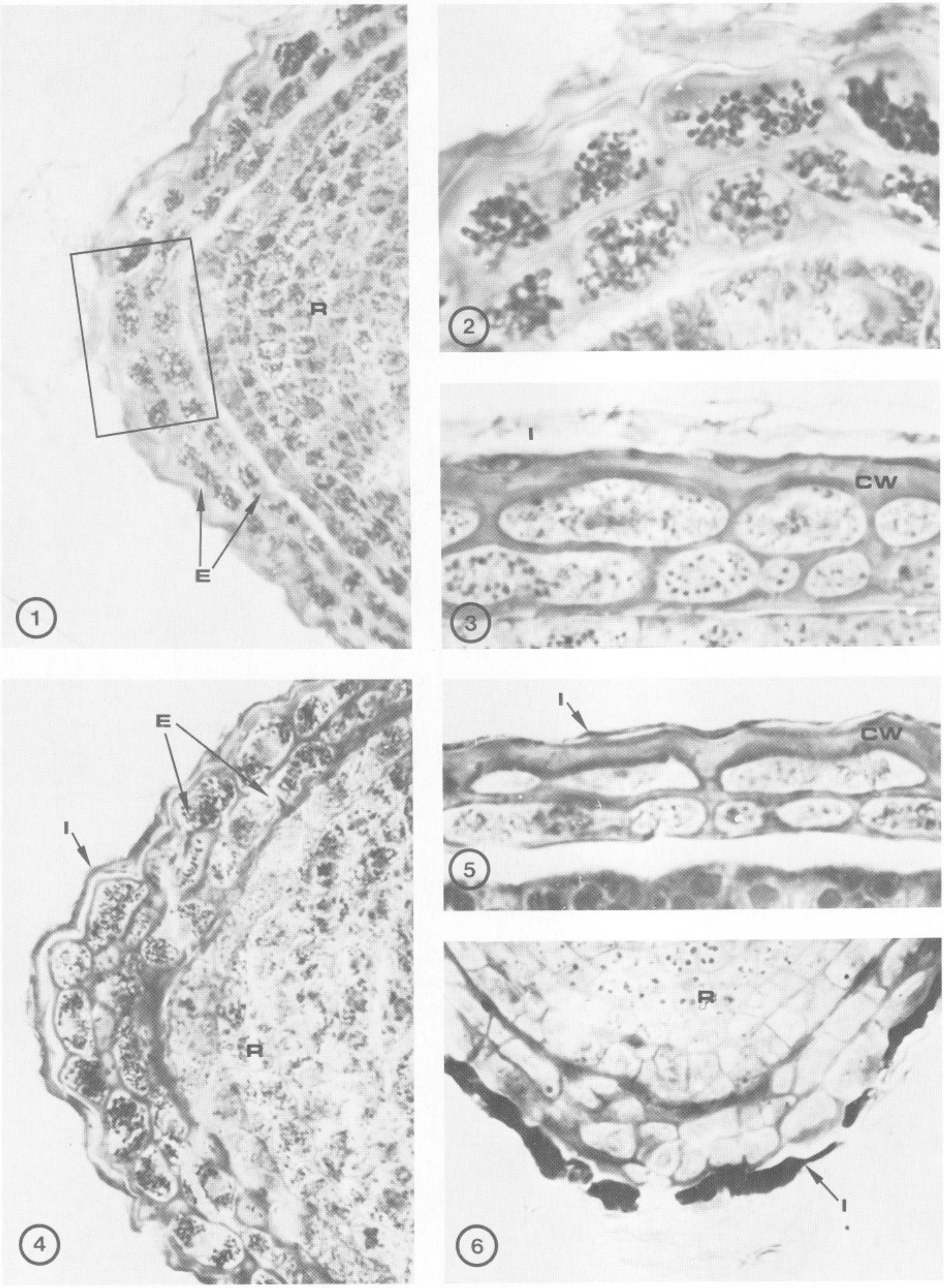
Discussion

The endosperm of lettuce achenes has been considered as a source of food for the growing embryo and as a mechanical restriction for radicle elongation. PARK and CHEN (1974) reported that the endosperm storage material is completely utilized during the first 3 days after germination is completed. According to our observations, the actual mobilization of endosperm cell wall materials does not occur before endosperm rupture. Biochemical work has shown that the major component of the endosperm cell walls is mannan (HALMER et al. 1975). Also, endosperm-stored reserves are mobilized after germination is completed (HALMER et al. 1978; HALMER and BEWLEY 1979), and breakdown of endosperm cell walls occurs between 15 and 25 h after sowing

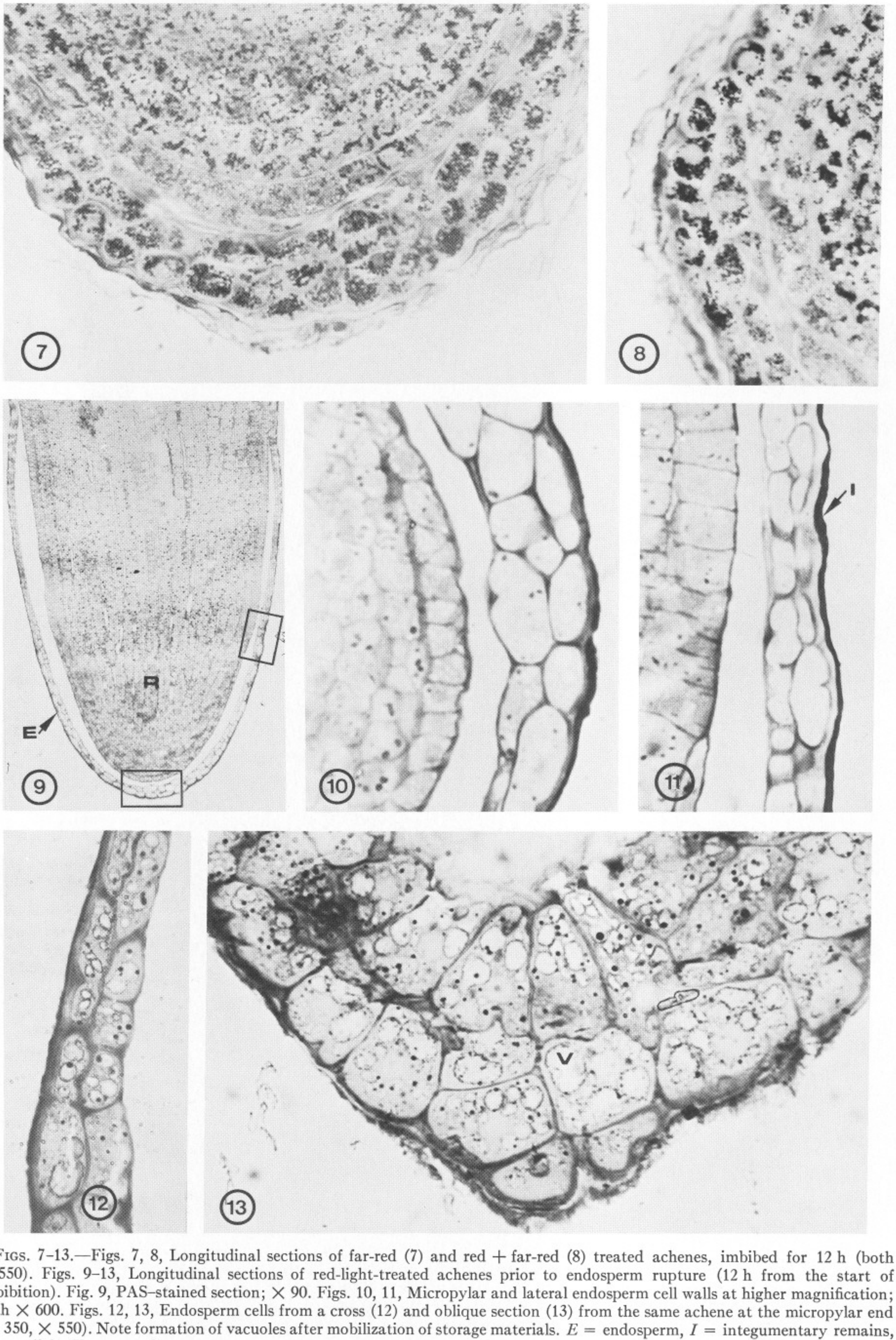
TABLE 1

PERCENTAGE OF RED-LIGHT-TREATED ACHENES EXHIBITING STRUCTURAL CHANGES OF ENDOSPERMS AT VARIOUS STAGES FROM THE START OF IMBIBITION

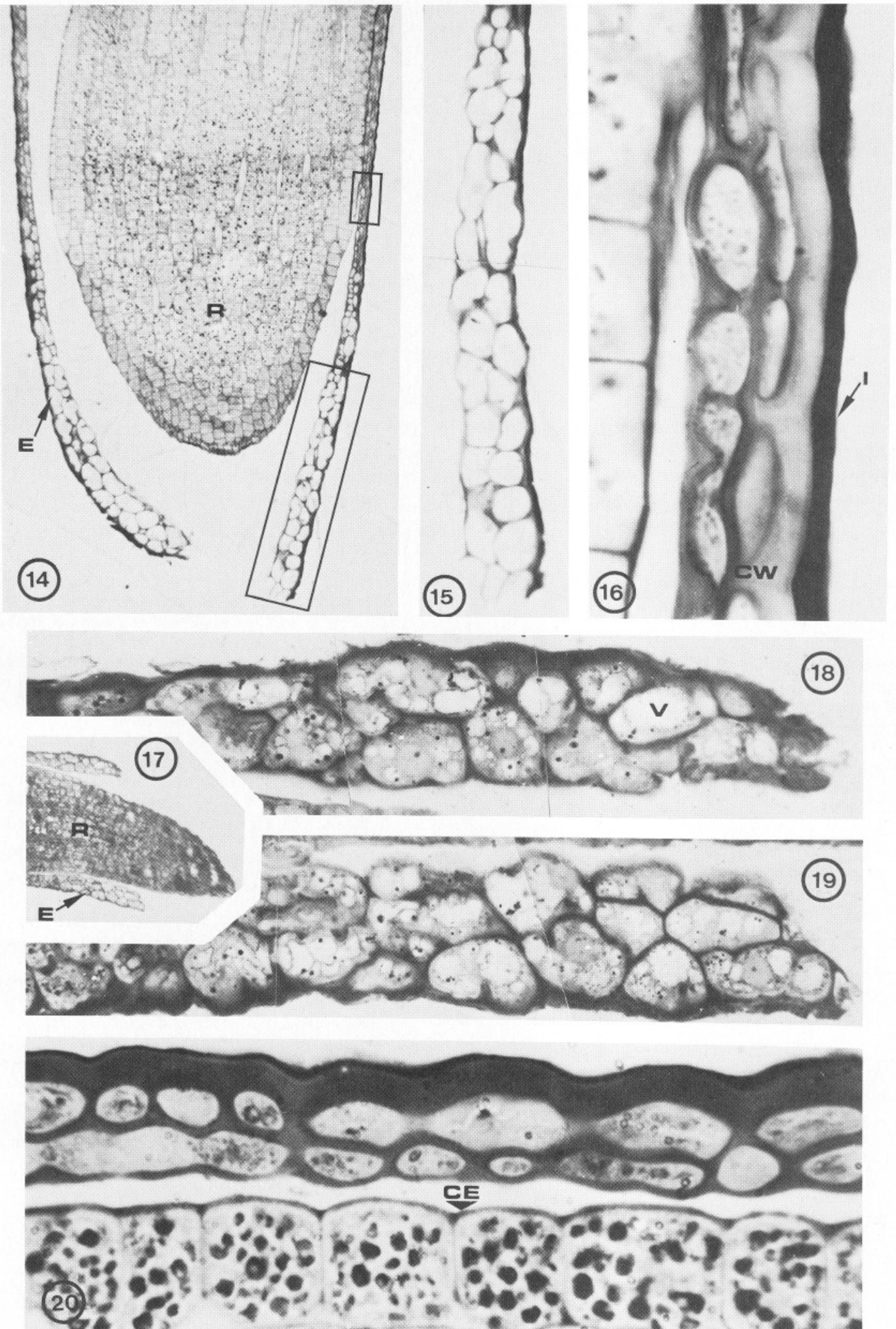
No. of hours	Endosperm unchanged	Endosperm showing structural changes	Endosperm ruptured
6	100	. . .	. . .
9	100	. . .	. . .
12	70	16	14
15	5	. . .	95



FIGS. 1–6.—Median longitudinal sections of achenes imbibed in darkness for 1 h (figs. 1–3, 6) and for 12 h (figs. 4, 5). Figs. 1, 4, 6, Structure of micropylar region after toluidine blue (1, 4) and PAS (6) staining; × 600, × 600, × 450. Figs. 2, 3, 5, Endosperm cells from micropylar (2) and lateral area (3, 5) at higher magnification; × 1,500. Note the thickness of cell walls, the abundance of storage materials, and the absence of an apparent vacuolar system from the endosperm cells. *CW* = cell wall, *E* = endosperm, *I* = integumentary remains, *R* = radicle.



FIGS. 7–13.—Figs. 7, 8, Longitudinal sections of far-red (7) and red + far-red (8) treated achenes, imbibed for 12 h (both × 550). Figs. 9–13, Longitudinal sections of red-light-treated achenes prior to endosperm rupture (12 h from the start of imbibition). Fig. 9, PAS–stained section; × 90. Figs. 10, 11, Micropylar and lateral endosperm cell walls at higher magnification; both × 600. Figs. 12, 13, Endosperm cells from a cross (12) and oblique section (13) from the same achene at the micropylar end (× 350, × 550). Note formation of vacuoles after mobilization of storage materials. *E* = endosperm, *I* = integumentary remains, *R* = radicle, *V* = vacuole.



FIGS. 14–20.—Red-light-treated achenes with ruptured endosperms. Figs. 14–16, PAS-stained section from an achene imbibed for 12 h (14, × 130). Fig. 15, Micropylar endosperm; × 300. Fig. 16, lateral endosperm; × 1,800. Note existence of cell wall material. Figs. 17–20, Achene imbibed for 15 h. Figs. 17–19, Micropylar area of the endosperm; note the absence of storage materials and the vacuolation of these endosperm cells; × 80, × 700, × 700. Fig. 20, Longitudinal section of lateral endosperm from the same achene. Note the thickness of the cell walls; × 1,900. *CE* = cotyledonary epidermis, *CW* = cell wall, *E* = epidermis, *I* = integumentary remains, *R* = radicle, *V* = vacuole.

(HALMER et al. 1978), which also is after germination is completed. The endosperm may be an early source of food reserves for the growing seedling, and the degradation process is promoted only indirectly by red-light illumination through stimulation of germination (HALMER et al. 1976).

Our observations agree with these biochemical data in that the actual mobilization of the materials of the entire endosperm does not occur prior to tissue rupture. We find that the cytoplasm of the micropylar endosperm cells undergoes drastic changes even before radicle protrusion, even though the cell walls of the entire endosperm still remain PAS positive for at least a short period after endosperm rupture and radicle elongation. JONES's (1974) observations on the disintegration of the endosperm cell walls were probably derived from postgerminative stages of the seedling development.

The structural changes of the endosperm of germinating *Lactuca sativa* achenes agree with the SEM observations of PAVLISTA and VALDOVINOS (1978) in relation to both alterations to the endosperm and timing.

The exact role of the endosperm and the mechanism of its rupture are still unknown (EVENARI 1965; MAYER and SHAIN 1974). Our light microscopic observations demonstrate that the endosperm cells of the micropylar end undergo characteristic changes prior to the opening of the tissue. In our opinion, these structural changes are not the consequence of of an exclusively mechanical action resulting from embryo expansion. Furthermore, endosperm rupture does not appear to be based on degradation or structural weakening of the entire tissue or of the cell walls at the micropylar end. We suggest that the changes in the micropylar endosperm area are a prerequisite for the rupture of the tissue and, therefore, for radicle protrusion.

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