

Immunocytochemical Localization of Wheat Germ Agglutinin in Wheat

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ABSTRACT Immunocytological techniques were developed to localize the plant lectin, wheat germ agglutinin (WGA), in the tissues and cells of wheat plants. In a previous study we demonstrated with a radioimmunoassay that the lectin is present in wheat embryos and adult plants both in the roots and at the base of the stem. We have now found, using rhodamine, peroxidase, and ferritin-labeled secondary antibodies, that WGA is located in cells and tissues that establish direct contact with the soil during germination and growth of the plant. In the embryo, WGA is found in the surface layer of the radicle, the first adventitious roots, the coleoptile, and the scutellum. Although found throughout the coleorhiza and epiblast, it is at its highest levels within the cells at the surface of these organs. In adult plants, WGA is located only in the caps and tips of adventitious roots. Reaction product for WGA was not visualized in embryonic or adult leaves or in other tissues of adult plants. At the subcellular level, WGA is located at the periphery of protein bodies, within electron-translucent regions of the cytoplasm, and at the cell wall–protoplast interface. Since WGA is found at potential infection sites and is known to have fungicidal properties, it may function in the defense against fungal pathogens.

Lectins bind and cross-link specific monosaccharides and oligosaccharides. They thus serve as powerful probes in the study of the carbohydrate moieties at cell surfaces (see reference 14 for review). Because this remarkable specificity is probably not fortuitous, the function of lectins in the organisms in which they are found should reflect their particular sugar-binding properties. In some cases the binding of a lectin to a specific carbohydrate receptor has been shown to mediate recognition events at the cellular level. Examples include influenza virus, which possesses a hemagglutinin that allows the virus to attach to sialic acid residues found on host cell plasma membranes (45), and the amoebae of *Dictyostelium discoideum*, which during aggregation synthesize a lectin that promotes cell-cell adhesion (1).

Although higher plants have been the richest source of lectins, the role of lectins in these organisms is poorly understood. An oft-repeated hypothesis is that lectins act as receptor sites for symbionts (3, 34) or pathogens (35). For example, there is evidence that suggests that in certain legumes lectins might be receptors for the nitrogen-fixing rhizobia that form symbiotic nodules in legume roots (3, 10, 34, 40). Other evidence, consistent with their cytotoxic properties and their high

concentration in certain seeds, suggests that they may serve as a defense against seed-eating herbivores (17).

Investigations such as these on the roles of lectins in plants have been confined largely to the legume family. Lectins, however, are a diverse group of proteins and glycoproteins that are found throughout the plant kingdom and, in all likelihood, perform diverse functions. We have sought, therefore, to broaden this focus by examining the tissue distribution of wheat germ agglutinin (WGA), a lectin from the grass family. Although a study such as this by itself will not discern the function of the lectin, it will establish criteria consistent with its role in the plant.

WGA is the most thoroughly characterized of the numerous lectins that bind *N*-acetylglucosamine (GlcNAc) and its biologically widespread polymer, chitin (see reference 14 for review). In addition, its toxic effects on fungal metabolism (24) suggest a role in the defense against pathogens.

In our previous study (25) we quantified the levels of WGA in wheat plants with a radioimmunoassay (RIA). We found that ungerminated wheat grains contain three orders-of-magnitude less lectin than do typical legume seeds, or $\sim 1 \mu\text{g}$ of WGA per grain, all associated with the embryo. After one

month of growth, WGA levels were found to be one-third to one-half of those in ungerminated embryos. In contrast, the vast reserves of lectin present in legume seeds largely disappear during the first few weeks of growth (16, 30, 31, 33, 42). In adult wheat plants, WGA is present at the base of the shoot in the nodal region and in the roots as well, especially in recently emerged adventitious roots (25). Here we used immunocytochemistry to localize WGA within the tissues and cells of wheat embryos and plants.

MATERIALS AND METHODS

Plants

Wheat grain, *Triticum aestivum* L. cv. "Era", was purchased from Olds Seed Company (Madison, Wis.). Embryos were dissected from grain that had imbibed in aerated deionized H₂O for 10 to 24 h. Older tissue was obtained from plants grown in the greenhouse in sand-filled clay pots kept moist with half-strength Hoagland's solution.

Antibody Preparation

The immunoglobulin fraction from rabbit antisera against WGA, obtained as described earlier (25), was affinity-purified on a WGA-Sepharose 4B column prepared by the cyanogen bromide method (22). Those immunoglobulins bound to the immobilized WGA through their GlcNAc or sialic acid residues were eluted from the column with phosphate-buffered saline (PBS) containing 100 mM GlcNAc. Since this nonantigenically-bound material most likely still contained some immunoglobulins specific to WGA, the material eluted with GlcNAc was combined with the column flow-through for subsequent reabsorption to the WGA-Sepharose after immunoadsorbed material had been removed by elution of the column with one column volume of 3 M MgCl₂ followed by PBS. This procedure was repeated four times. The immunoadsorbed material, designated AS, was dialyzed extensively against PBS and stored at -20°C in the presence of 0.02% sodium azide. Antibody freed of specific anti-WGA by four passes through WGA-Sepharose as described above was similarly dialyzed and used as control serum (CS). Stock solutions of AS and CS were prepared to an A₂₈₀ of 1.3 and were diluted similarly for experimental use. Immunoelectrophoresis was performed as described previously (25).

Tissue Preparation

Embryos obtained from imbibed grain or tissue excised from plants were fixed overnight at 4°C in 4% formaldehyde (Polysciences, Inc., Warrington, Pa.) in PBS. Samples were brought to room temperature, extensively washed in PBS, and placed in 25% polyvinylpyrrolidone (PVP-40, Sigma Chemical Co., St. Louis, Mo.; wt/vol in PBS) for 30 to 60 min. The PVP, purified by dialysis as described by Skaer et al. (36), served as a cryoprotectant that significantly enhanced the quality of tissue preservation. Tissue was transferred directly from the PVP into a plastic capsule (Electron Microscopy Sciences, Fort Washington, Pa.) filled with O.T.C. compound (Lab-Tek Products, Naperville, Ill.) and frozen in liquid *n*-hexane that was in equilibrium with its solid phase. The blocks were attached to stubs by freezing and sectioned at -15°C in a cryostat (Model CTI, International Equipment Co., Needham Heights, Mass.). Sections 6-8 μm in thickness were picked up with a wire loop and placed in 3% formaldehyde and 0.3% glutaraldehyde (Polysciences, Inc.) in PBS at 4°C. All further steps were conducted at room temperature. Fixative was removed and the sections were washed extensively (six changes of buffer, 5 ml each) in PBS containing 10 mM glycine. Glycine was included in the PBS in all further steps to eliminate any unreacted aldehyde groups. Plant tissue is rich in various moieties with nonantigenic affinity for immunoglobulins. To ensure that these sites would not be available to the primary or secondary antibodies, the cryostat sections were treated with undiluted nonimmune goat serum as described below.

Immunofluorescence

Sections were treated with whole, undiluted goat serum for 15 min. The serum was drawn off and 100 μl of 32-fold diluted AS or CS was applied. The sections were incubated for 40 to 60 min and then washed for a total of 30 min with four changes of PBS. Goat serum was applied as before, drawn off, and the sections were incubated for 30 min with 32- or 64-fold diluted rhodamine-conjugated goat anti-rabbit immunoglobulin (Miles Laboratories, Elkhart, Ind.). Sections were washed as described above and mounted on glass slides.

The sections were viewed with a Reichert Ultrastar microscope (American Optical Corp., Buffalo, N. Y.) equipped for differential interference contrast

(DIC) and epifluorescence optics using mercury vapor illumination coupled with appropriate filters for rhodamine fluorescence. Images were photographed on Tri-X film (Eastman Kodak, Rochester, N. Y.) which was then developed in HC110 dilution D (Kodak) with Factor 8 (MinMax, No. Hollywood, Calif.) added to enhance sensitivity.

Immunoperoxidase

The following steps were carried out to inhibit the abundant peroxidase activity of the wheat tissue. Sections were dehydrated in a graded methanol series and incubated for 30 min in 100% methanol containing 1% hydrogen peroxide (vol/vol). Sections were then rehydrated in a graded methanol series, incubated with phenylhydrazine hydrochloride (Sigma Chemical Co.; 1% in PBS, wt/vol; 41) for 1 h, and finally washed extensively in PBS. The sections were then treated with goat serum as described above and incubated for 1 h in AS or CS diluted 64-fold in PBS. Sections were washed in PBS for 30 min and then incubated for 30 min in goat anti-rabbit immunoglobulin diluted 80-fold in whole goat serum. After another 30-min wash, the sections were incubated with peroxidase-anti-peroxidase (PAP) complex (Miles Laboratories) diluted 60-fold in whole goat serum. Sections were washed as before and reaction product was developed for 5 to 7 min in diaminobenzidine tetrahydrochloride (Sigma Chemical Co.; 0.5 mg ml⁻¹ in 0.05 M Tris buffer, pH 7.6, containing 0.01% (vol/vol) hydrogen peroxide). Sections were washed extensively in deionized water, mounted on glass slides, and viewed with a Reichert Zetopan microscope equipped with differential interference contrast optics.

Immunoferritin

Cryostat sections (12 μm) were prepared, and as outlined above, incubated with goat serum followed by 64-fold diluted AS or CS, and washed in PBS. The sections were then incubated for 30 min with ferritin-conjugated goat anti-rabbit immunoglobulin (Miles Laboratories) diluted 128-fold in whole goat serum. After they were washed extensively in PBS (six changes in rapid succession, followed by another wash for 4 h at 4°C and a final wash overnight at 4°C), the sections were fixed for 15 min in 3% glutaraldehyde in 0.02 M phosphate buffer, pH 6.8. They were then postfixed in 2% osmium tetroxide, dehydrated, and embedded in Spurr's resin (39). To facilitate handling, the sections were suspended in warm 1% agar after they had been fixed in 3% glutaraldehyde. After the agar had solidified, small blocks containing single sections were excised for further processing.

Since the ferritin-labeled antibody penetrates only a short distance into the tissue, care was taken to obtain ultrathin sections from the surface of the plastic-embedded cryostat sections. The ultrathin sections were viewed either unstained or poststained in 2% aqueous uranyl acetate with a Zeiss 10A electron microscope operated at 60 kV.

RESULTS

Antibody and Tissue Preparation

The immunoglobulin fraction from the anti-WGA serum used in the RIA studies (25) was affinity purified as described in Materials and Methods for immunocytochemistry. On immunoelectrophoresis against purified WGA or crude wheat germ extract, the purified anti-WGA displayed a single precipitin arc without spurs (Fig. 1). The final column flowthrough

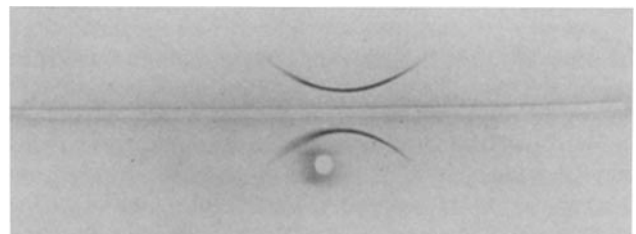


FIGURE 1 Immunoelectrophoresis of purified WGA (top well) and crude wheat germ extract (bottom well). 2.5 μg of WGA was placed in each well. After electrophoresis, affinity-purified anti-WGA was added to the trough and allowed to diffuse overnight. Before it was photographed, the gel was stained with Coomassie Blue. Note that single precipitin arcs formed when the AS diffused against either purified WGA or the crude wheat germ extract.

and final GlcNAc wash of the WGA-Sepharose column were combined and utilized as CS. Routinely, near-adjacent cryostat sections were incubated with similarly diluted AS or CS. With the protocol described in Materials and Methods, nonspecific staining of controls was observed only at low levels. An alternative control (not shown) performed with an IgG fraction prepared as described earlier (25) from nonimmune rabbits yielded results indistinguishable from those obtained with CS. In addition, a localization pattern identical to the one obtained with AS was seen with a rabbit anti-WGA immunoglobulin fraction prepared by Vector Laboratories, Inc. (Burlingame, Calif.; data not shown).

Because autofluorescence in the grain is restricted to the coat, it did not pose a problem in visualizing specific antibody fluorescence in embryos. However, after the grain germinates, additional strong autofluorescence indicative of phenolic acids or lignin (37) is evident, especially in the secondary cell walls of vascular tissue (not shown). Because this autofluorescence tended to obscure the fluorescence from rhodamine- or fluorescein-labeled antibodies, a peroxidase-based method was developed to survey older plants. Localization by peroxidase was possible only after a procedure was developed to inhibit the abundant peroxidases found in wheat tissue. Neither H₂O₂ and methanol nor phenylhydrazine treatments alone (41) adequately reduced background reaction product. However, satisfactory localization was achieved when these procedures were applied in sequence as described in Materials and Methods. The specificity of the PAP technique was compared to that of immunofluorescence, using the wheat embryos. Both procedures were found to yield identical localization patterns (compare Figs. 2B and 4A).

Localization of WGA in Wheat Embryos

The wheat embryo is appressed to the endosperm at one end of the grain (Fig. 2A). It contains the primordial organs of the adult plant (leaves, radicle, the first adventitious roots), layers of cells that ensheath these organs (coleoptile and coleorhiza), and an organ that absorbs nutrients from the starchy endosperm as the embryo germinates and begins to grow (scutellum).

Wheat embryos display little autofluorescence when illuminated with 530-nm light and viewed through the appropriate barrier filters for the visualization of rhodamine fluorescence. Although the grain coat is strongly autofluorescent (Fig. 2D), this tissue contains little WGA as determined by RIA (25). Similar autofluorescence is observed when the grain coat is stimulated by blue light, conditions (Fig. 2C) which do not excite the specific rhodamine fluorescence described later, but which are known to induce autofluorescence in other plant tissues (37).

WGA appears to be specifically located in several of the embryo organs and cell layers described above. The bulk of the reaction product for WGA is located in the embryonic roots and their ensheathing tissue, the coleorhiza. In the radicle, WGA is limited largely to the surface layer of cells (Fig. 2B) and to the root cap, except for label sometimes seen in one or two files of cells in the center of the root (Fig. 2B). Similarly, the first adventitious roots, when cut in cross-section (Fig. 3), display reaction product mainly in the layer of cells at the root surface (Fig. 3A inset and C). In the coleorhiza, lectin is more generally distributed and occurs in all cells (Fig. 4C). However, the exterior layer of cells stains more intensely than those in the interior of this organ (Fig. 4C). A staining pattern similar

to that in the coleorhiza is found in the epiblast (Fig. 4B). In all sections incubated with CS or nonimmune IgG, no specific reaction product was found (Figs. 2D, 3D, 4D and E, and 5B and E).

The embryonic leaves do not appear to contain WGA (e.g., Fig. 4A and 5A). However, the cells on the exterior surface of the coleoptile stain densely for WGA (Fig. 5A and C). Cells on the surface of the coleoptile that faces the shoot occasionally exhibit low levels of reaction product (not shown) while those in the interior of this organ appear to lack WGA (Fig. 5A and C). The lectin is absent from the scutellum, except in those cells on the surface opposite the coleoptile (Fig. 5A, arrowhead). Similarly, the single layer of cells that lines the interior surface of the grain coat displays reaction product (Fig. 5D and E). In summary, large regions of the embryo are devoid of specific reaction product, while its external surfaces are covered by cells that contain WGA.

Localization of WGA in Adult Plants

RIA of extracts of wheat plants up to one month in age detected WGA only at the base of the shoot and in the roots (25). The amount of lectin in the shoot base remains at ~300 ng of WGA per plant throughout the first month of growth. Furthermore, recently emerged adventitious roots are approximately 20-fold enriched in lectin as compared to older portions of the root system (2% and 0.1% of the extracted protein, respectively; 25).

Immunocytochemistry reveals that the lectin in the shoot base of two-month-old plants is located in unemerged adventitious roots. The cap and outer layers of these roots appear to contain WGA (Fig. 6A). These structures retain the lectin as the root expands (Fig. 6C) and breaks through the stem epidermis (Fig. 6E). In cross-section, reaction product is most evident in the root cap cells ensheathing the root (Fig. 6G) but is also present, though less prominently, in the outer layers of the root itself (Fig. 6G, arrowhead). Control sections showed no reaction product (Fig. 6B, D, F, and H).

Localization at the Subcellular Level

At the light microscope level, rhodamine and peroxidase labels for WGA appear to be located in the cytoplasm, often in particulate inclusions (Figs. 5C and 7), and in the cell wall. Because the particulate inclusions stain when the sections are treated with modifications of the protein histochemical techniques (eq. 12), they appear to be protein bodies. To identify definitively the sites of WGA within cells, localization at the ultrastructural level was performed. For ultrastructural studies, the AS- or CS-treated cryostat sections were incubated with ferritin-labeled second antibody and embedded for electron microscopy. Of the tissues that contain WGA, we selected the cells at the surface of the radicle and the coleoptile as representative material for ultrastructural examination.

In the radicle (Figs. 8 and 9A) and coleoptile (Fig. 9C), immunospecific ferritin grains are located predominantly within inclusions whose distribution and size suggest that they are equivalent to the protein bodies visualized at the light microscope level. In unstained sections (Fig. 8) as well as in those poststained with uranyl acetate (Fig. 9A and C), most of the ferritin is found adjacent to the inner surface of the organelle. The majority of the protein body matrix lacks antibody-specific sites. To a lesser extent, WGA is found in the cytoplasm (Fig. 8B, stars), mostly at the periphery of electron-lucent regions. These regions may be the remnants of mem-

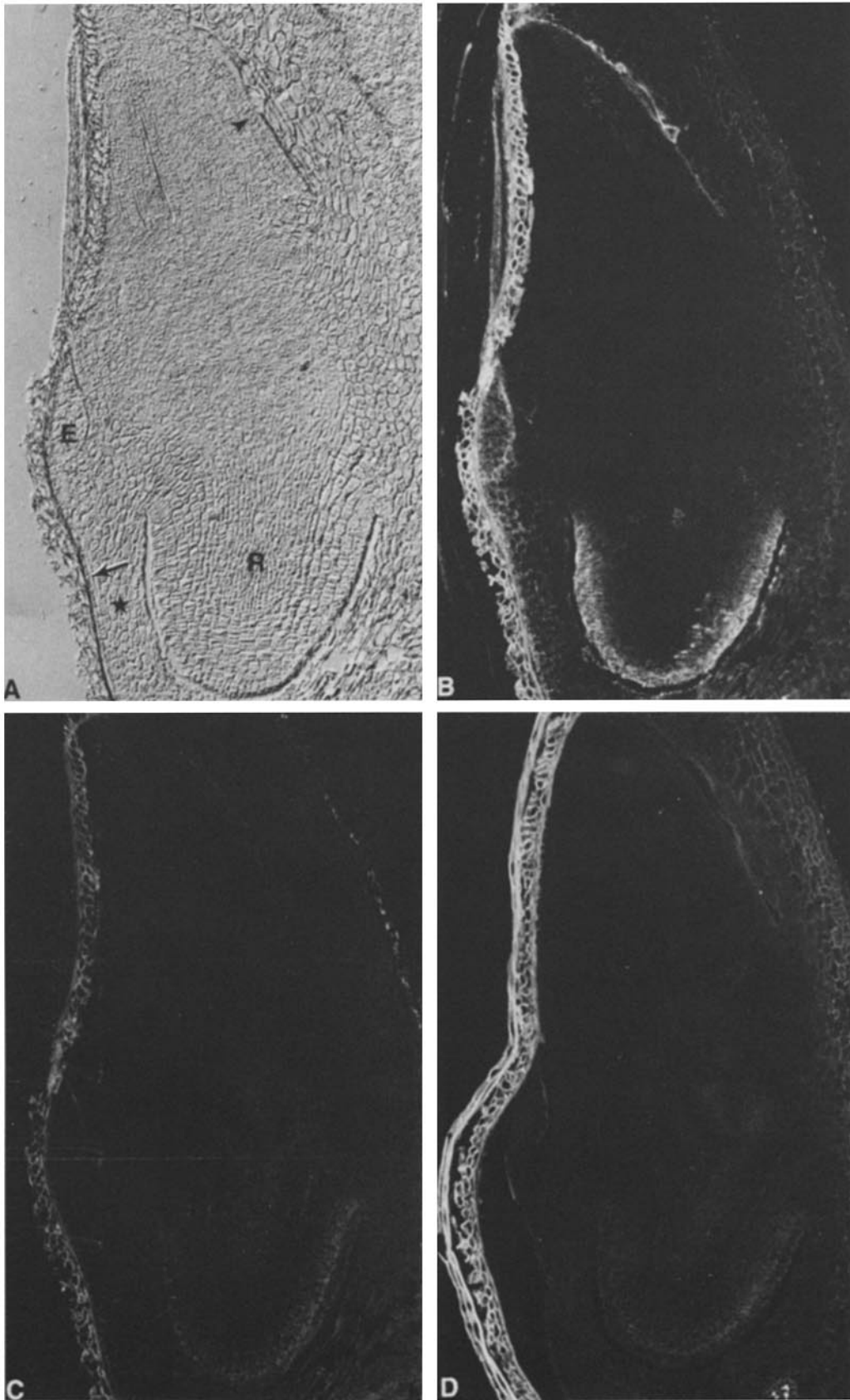


FIGURE 2 Near-median longitudinal sections of a wheat embryo. *A*, *B*, and *C* depict the same section photographed under different conditions; *D* is a near-adjacent section to the one depicted in *A*, *B*, and *C*. Sections were treated with AS (*A*, *B*, and *C*) or CS (*D*) followed by rhodamine-conjugated goat anti-rabbit immunoglobulin. They were photographed with differential interference contrast optics (*A*) and with fluorescence optics using green excitation (*B* and *D*) for concomitant visualization of grain coat autofluorescence and specific rhodamine fluorescence, or blue excitation (*C*) for visualization of coat autofluorescence alone. Antibody-linked fluorescence is evident in the AS- (*B*) but not in the CS-treated (*D*) sections. Note the specific fluorescence in *B* associated with the radicle, epiblast, coleorhiza, and coleoptile. Isolated cells in the center of the radicle also display specific fluorescence. Grain coat autofluorescence is visible with either green (*B* and *D*) or blue (*C*) light excitation. Radicle (*R*), grain coat (arrow), epiblast (*E*), coleoptile (arrowhead), and coleorhiza (star). $\times 75$.

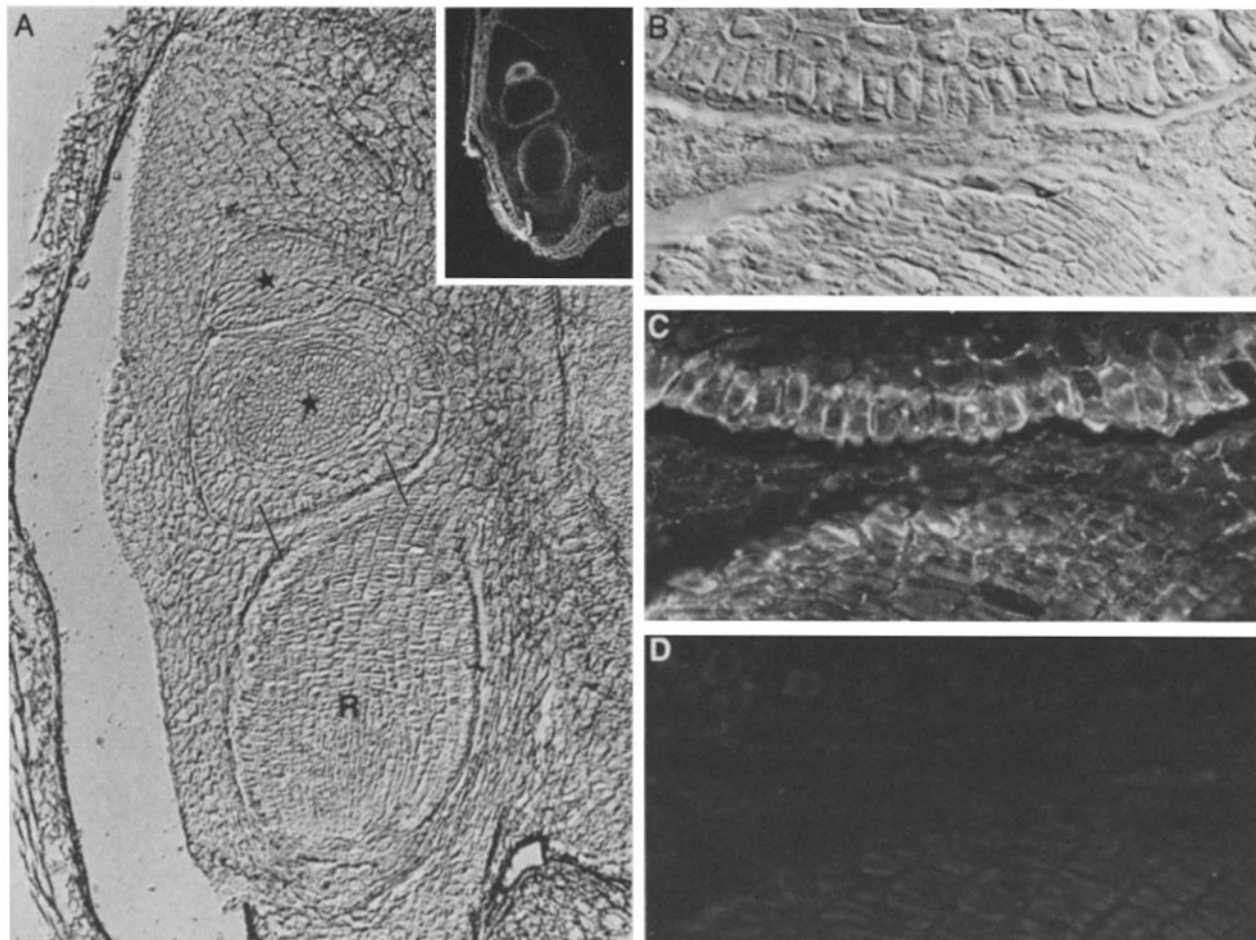


FIGURE 3 Longitudinal section through the side of a wheat embryo. The radicle (*R*) is cut in glancing section while the first two adventitious roots (stars) are cut in cross-section. The central region of the embryo (bracketed in *A*) is shown at higher magnification in *B*, *C*, and *D*. The *inset* to *A* depicts a near-adjacent section to that shown at higher magnification in the same panel. In the *inset*, specific immunofluorescence is visible at the periphery of the radicle, which was cut longitudinally, and in an adventitious root, which was cut in cross-section. The tip of another adventitious root displays specific reaction product as well. The autofluorescent grain coat surrounds the section. Note that fluorescence for WGA is most intense at the surface of the roots (*C*). A similar section treated with CS displays no specific fluorescence (*D*). The sections were photographed with DIC optics (*A* and *B*) or with fluorescence optics (*A inset*, *C*, and *D*). *A*: $\times 80$; and *inset*: $\times 18$. *B*, *C*, *D*: $\times 230$.

brane-bounded vesicles or endoplasmic reticulum cisternae whose limiting membranes were lost during freezing, fixation, or embedment. WGA is also found at the interface between the cell wall and the protoplast (Fig. 10).

Ultrathin sections cut from the surface of cryostat sections that had been incubated with CS and ferritin-conjugated second antibody lacked reaction product (Figs. 9*D* and 10*B*). As an alternate control, cells in the interior of the radicle on cryostat sections that had been treated with AS were checked for reaction product. Since these cells, as well as those at the surface of the radicle, were exposed at the surface of the cryostat sections, both had equal access to antibody. Despite the similarity in ultrastructure of the outer cells and those six to seven layers to the interior of the radicle (i.e., they are both rich in protein bodies and lipid inclusions), no reaction product is evident within the interior cells (Fig. 9*B*). Thus, it is unlikely that the ferritin seen in surface cells represents nonspecific binding of antibodies to cellular components.

DISCUSSION

The results reported here, as well as our previous findings (25), demonstrate that WGA is heterogeneously distributed in wheat

embryos and plants. Particular organs, and specific cell layers within organs, contain the lectin. The consistent pattern that emerges from this study is that, in both embryos and adult plants, WGA is limited to regions where cells, initially enclosed by protective layers, break through these layers and gain direct exposure to the soil.

The coleorhiza and coleoptile are the first organs to emerge from a germinating embryo. Both structures contain WGA in their surface cells. Within the first 48 h of growth, the radicle and first adventitious roots penetrate the coleorhiza. As a result, both the internal cells of the coleorhiza and the surface cells of the roots are exposed to the soil. Each of these cell types also contains WGA. For the first 3–4 d after germination, growth of the coleoptile accompanies that of the shoot. This pattern is unlike that of the coleorhiza which, by the end of the second day of growth, is penetrated by the root. Thus, the shoot does not emerge from the coleoptile until it is 2–3 cm above the surface of the grain. The external layer of the coleoptile contains WGA, but neither its internal cells nor those on the surface of the first leaves display reaction product. It may be significant that the latter regions do not come into direct contact with the soil as do both the coleorhiza and the surface cells of the root.

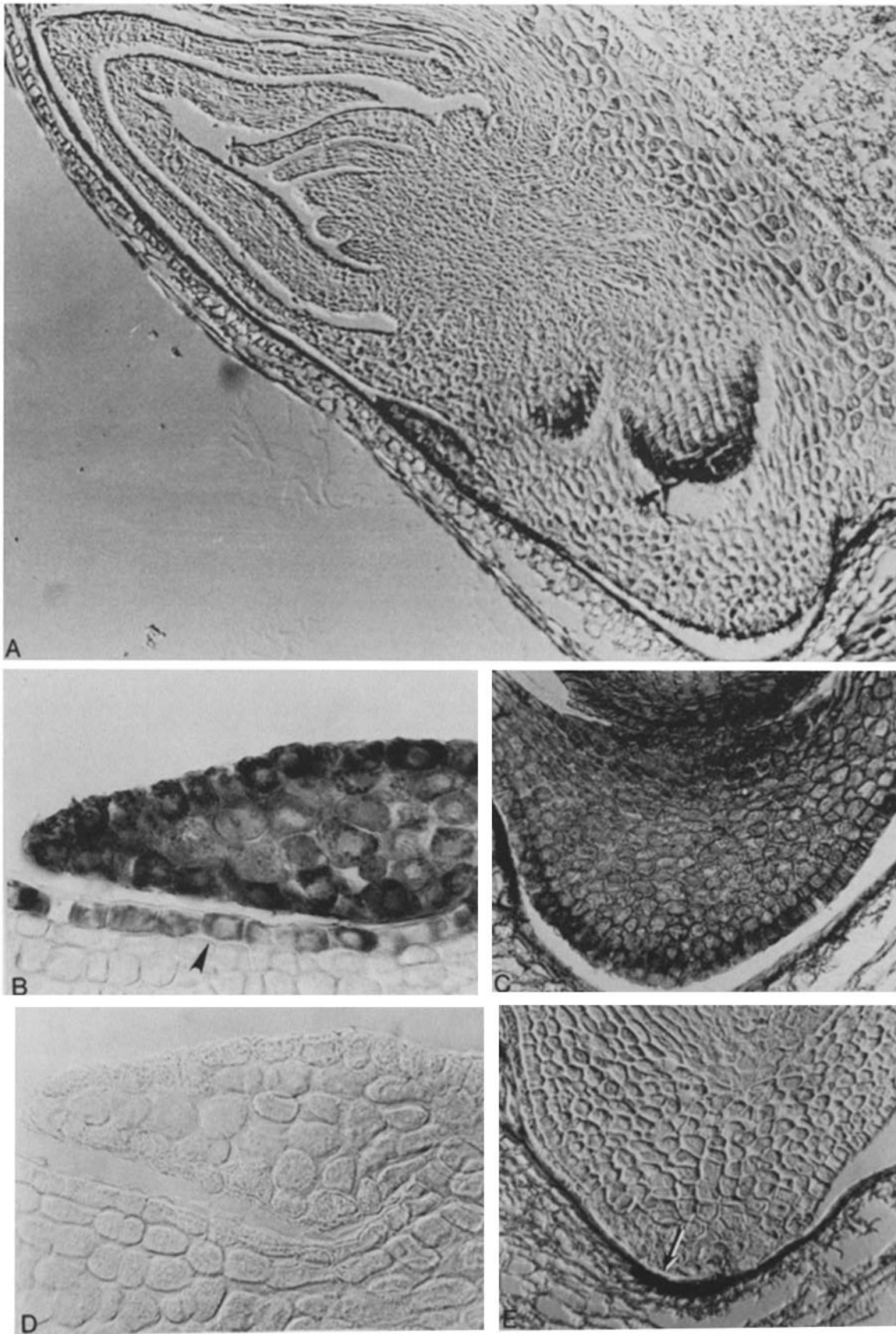


FIGURE 4 Near-longitudinal section through a whole wheat embryo (A) and sections through the epiblast (B and D) and the coleorhiza (C and E). The sections, photographed with DIC optics, were treated with AS (A, B, and C) or CS (D and E). Sites of antibody binding were visualized with the PAP technique. Compare the localization in A with that performed using fluorescence (Fig. 2 B). Note that, in contrast to the roots where it is mainly the surface cells that are labeled, all of the cells in the epiblast (B) and coleorhiza (C) contain WGA-specific reaction product. Also note, however, that reaction product is most dense in the surface cells of these organs. Surface cells of the coleoptile, which also stain for WGA (see Fig. 5), are visible in B (arrowhead). The dark layer of the grain coat (E, arrow) is naturally pigmented; hence, the darkened appearance of this area is not due to peroxidase reaction product. A: $\times 100$. B and D: $\times 300$. C and E: $\times 150$.

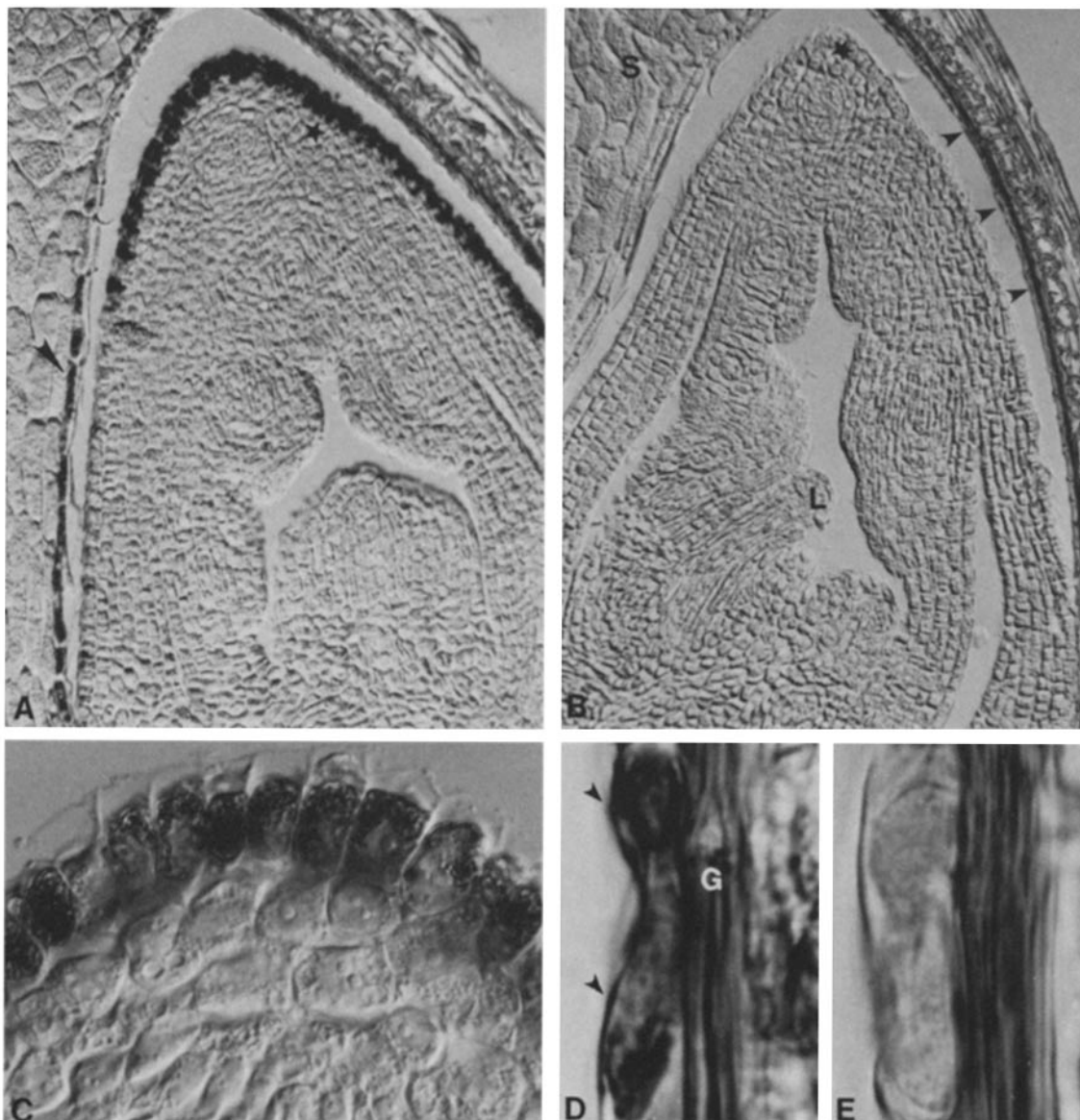


FIGURE 5 Sections through the leaves (*L*), coleoptile (star), and scutellum (*S*) of wheat embryos treated with AS (*A*, *C*, and *D*) or CS (panels *B* and *E*). The antigen-binding sites were visualized with the PAP technique, and the sections were photographed with DIC optics. Note in *A* that reaction product is largely limited to the external surface of the coleoptile (star) and scutellum (arrowhead). A higher-magnification image (*C*) shows that reaction product at the surface of the coleoptile is limited to the external cell layer. Cells adherent to the internal surface of the grain coat (small arrowheads in *B* and at higher magnification in *D* and *E*) also stain for WGA. Note that the grain coat (*G*, in *D*) is naturally pigmented and appears dark in AS, CS (*A*, *B*, *D*, and *E*), and untreated sections (not shown). *A* and *B*: $\times 140$. *C*: $\times 450$. *D* and *E*: $\times 1,575$.

WGA is present in adult plants in regions where developmental events take place analogous to certain events that occur in the embryo. Just below the epidermis, at nodes near the base of the shoot, adventitious roots are initiated, differentiate, and eventually emerge through the surface of the shoot. This temporal sequence is accompanied by a WGA histological pattern similar to that of the embryo, i.e., lectin is located on the surface of the new root and on the root cap.

Both the amount and localization of WGA differ substantially from that of the legume lectins so far examined. The highest amounts (1–4 mg per seed; 30, 31, 42) of legume seed lectins have been found in the cotyledons, the storage organs of the seed. Lectin is also present elsewhere in the embryonic plant axis, though at considerably lower levels (<1% of that in the cotyledons; 16, 23, 30, 31, 33, 42). Cytochemical studies

demonstrate that the seed lectins from the legumes *Canavalia ensiformis* (concanavalin A [Con A]; 9) and *Phaseolus vulgaris* (phytohemagglutinin; 23) are uniformly distributed throughout the cotyledons of these plants. In contrast to the legumes, wheat contains only $\sim 1 \mu\text{g}$ of lectin per grain (25, 43). Unlike the legume lectins, WGA is restricted to certain organs of the embryo and often to particular cell layers within these organs. Notably, WGA is absent from the storage tissue of the grain, the endosperm (25, 43).

In the legumes, the lectin levels decline rapidly during germination and the first few weeks of growth. In certain studies lectin was not detected in adult plants (16, 30, 33), except in developing seeds (16). Immunological techniques, however, have demonstrated that low levels of lectin similar to that of the seed are present in the roots of *Glycine soja* (40),

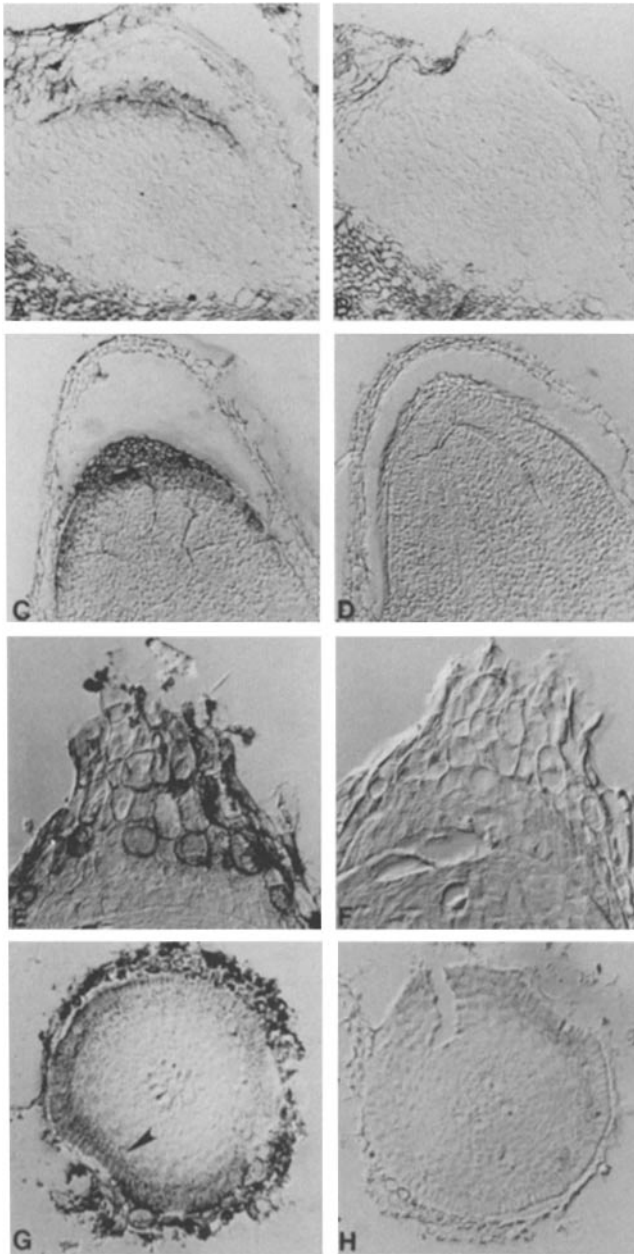


FIGURE 6 Sections of adventitious roots from 2-month-old wheat plants incubated with AS (A, C, E, and G) or CS (B, D, F, and H). In longitudinal sections of either recently formed (A and B) or expanding adventitious roots that have not yet emerged from the epidermis (C and D), reaction product for WGA is evident on the root cap and surface. In fully emerged roots cut in either longitudinal (E and F) or cross-section (G and H), reaction product is largely confined to the root cap, with more diffuse staining in the outer cell layer of the root tip (G, arrowhead). Antibody-binding sites were revealed with the PAP technique, and the sections were photographed with DIC optics. A, B, E, and F: $\times 75$. C and D: $\times 50$. G and H: $\times 100$.

Trifolium sp. (10), and *Pisum sativum* (13). Proteins that immunologically cross-react with antibodies to seed lectins have been found in other organs of adult plants but they do not possess hemagglutinating activity (23, 42).

In wheat, although the amount of WGA declines during germination and early growth, the levels remain at one-third to one-half of those in ungerminated grains throughout the first month of growth (25). The lectin in adult plants is similar

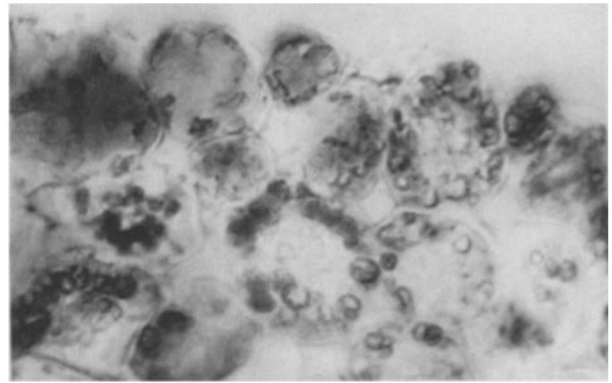


FIGURE 7 Section through the epiblast of a wheat embryo treated with AS. Antibody-binding sites were revealed with the PAP technique. Note that the reaction product for WGA is largely confined to vesicles within the cytoplasm. The section was photographed with DIC optics. $\times 750$.

to the grain-derived lectin, in that it cross-reacts with antibody, possesses hemagglutinating activity, and binds chitin (25). In adult plants, WGA is found only in the tips of adventitious roots, both before they emerge from the plant axis and afterward. Although lectin in adult soybean plants is also found in the roots, it is mainly present in the root hair region rather than at root tips (40).

At the intracellular level, the seed lectins from *Glycine max* (15) and *Ricinus communis* (44, 47) as well as WGA are found in the matrix of protein bodies. In addition, there are reports consistent with a similar location for phytohemagglutinin (23), Con A (9), and the lectin from *Datura stramonium* (19). Ultrastructural evidence indicates that the seed lectin from *Glycine max* is uniformly distributed throughout the matrix of protein bodies (15). WGA, however, is limited in its distribution within protein bodies to a zone in close proximity to the organelle membrane. The presence of WGA in wheat grains at a level three orders-of-magnitude lower than that of typical legume seed lectins (25, 43) is consistent with its limited distribution within protein bodies (i.e., at the organelle periphery).

These findings are consistent with results concerning the synthesis of WGA. WGA appears late during embryogenesis, at a time after protein bodies are filled with storage protein (43). Perhaps the lectin attains its peripheral location by binding to sugar receptors present near the organelle membrane. Since WGA is known to self-associate at sugar-binding sites (46), lectin-lectin aggregates may be present as well. Evidence from embryos and growing plants indeed indicates that WGA is bound at its sugar-combining site. For example, WGA is extracted from wheat germ most efficiently at low pH. Under these conditions, the dimer form of the lectin dissociates and the protein no longer binds its hapten (27). In addition, although small amounts of WGA are washed from the surface of intact roots in the absence of hapten, the presence of hapten greatly enhances the removal of lectin (25).

Recently, Bowles (5) has suggested that the reversible interaction of lectins through their sugar-binding sites with endogenous glycosylated receptors may regulate the activity of either the lectins or the receptors. In this context, specific endogenous receptors for soybean lectin and Con A were purified (6). The evidence presented here concerning WGA as well as reports on the lectins from clover (10) and the cellular slime molds (2, 38) indicate that there might be endogenous receptors for these lectins as well.

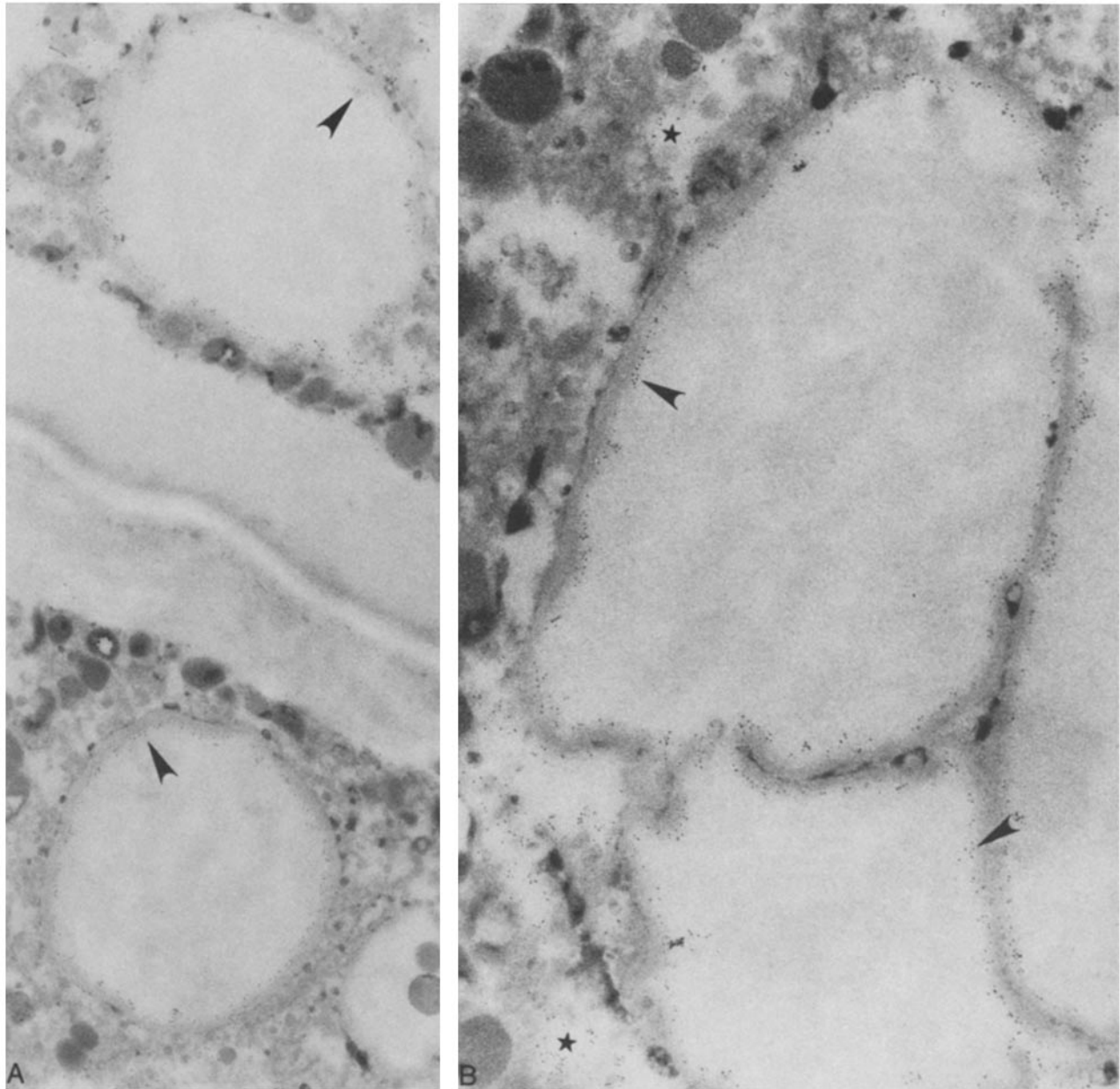


FIGURE 8 Electron micrographs of a radicle incubated with AS followed by ferritin-labeled goat anti-rabbit immunoglobulin. The tissue contains large numbers of protein bodies (see text) that appear as single (A) or multiple (B) units. Note that ferritin is largely confined to the periphery of the protein bodies (arrowheads). This is particularly evident at higher magnification (B). Some ferritin is found also in the cytoplasm, in association with electron-lucent areas (star). Sections were not poststained. A: $\times 34,000$. B: $\times 60,000$.

The WGA located at the root surface might be secreted from the cytoplasm as a result of an exocytic event in which protein bodies or protein body-derived vesicles fuse with the plasma membrane. The WGA we observe near the cell wall in embryonic root cells is perhaps the product of a small number of such events that might occur within a few hours of the beginning of imbibition. A detailed study of the synthesis and secretion of WGA from adventitious roots is currently in progress in our laboratory.

Lectins from a great diversity of organisms have also been localized to the cell surface or extracellular sites. For example, mucin cells from the marine sponge *Geodia cydonium* synthesize and secrete a galactose-specific lectin that is found on the surface of these and other cells in this organism (26). In another

example, aggregating amoebae of the cellular slime molds synthesize species-specific lectins that are thought to mediate aspects of cell-cell adhesion events (see reference 1 for recent review). Observations in support of this hypothesis include a cell surface location as demonstrated by immunocytochemistry (8) and cell surface iodination (38) of a small proportion of the lectin present in aggregating cells, a tenfold increase in surface lectin levels elicited by divalent ligands (38), and a mutant with an impaired lectin that is unable to proceed beyond the early stages of aggregation (32).

An example from the vertebrates is a lactose-specific lectin in chicks the appearance of which during development coincides with the time of greatest myoblast fusion activity (see reference 1 for review). Although a cell surface location for at

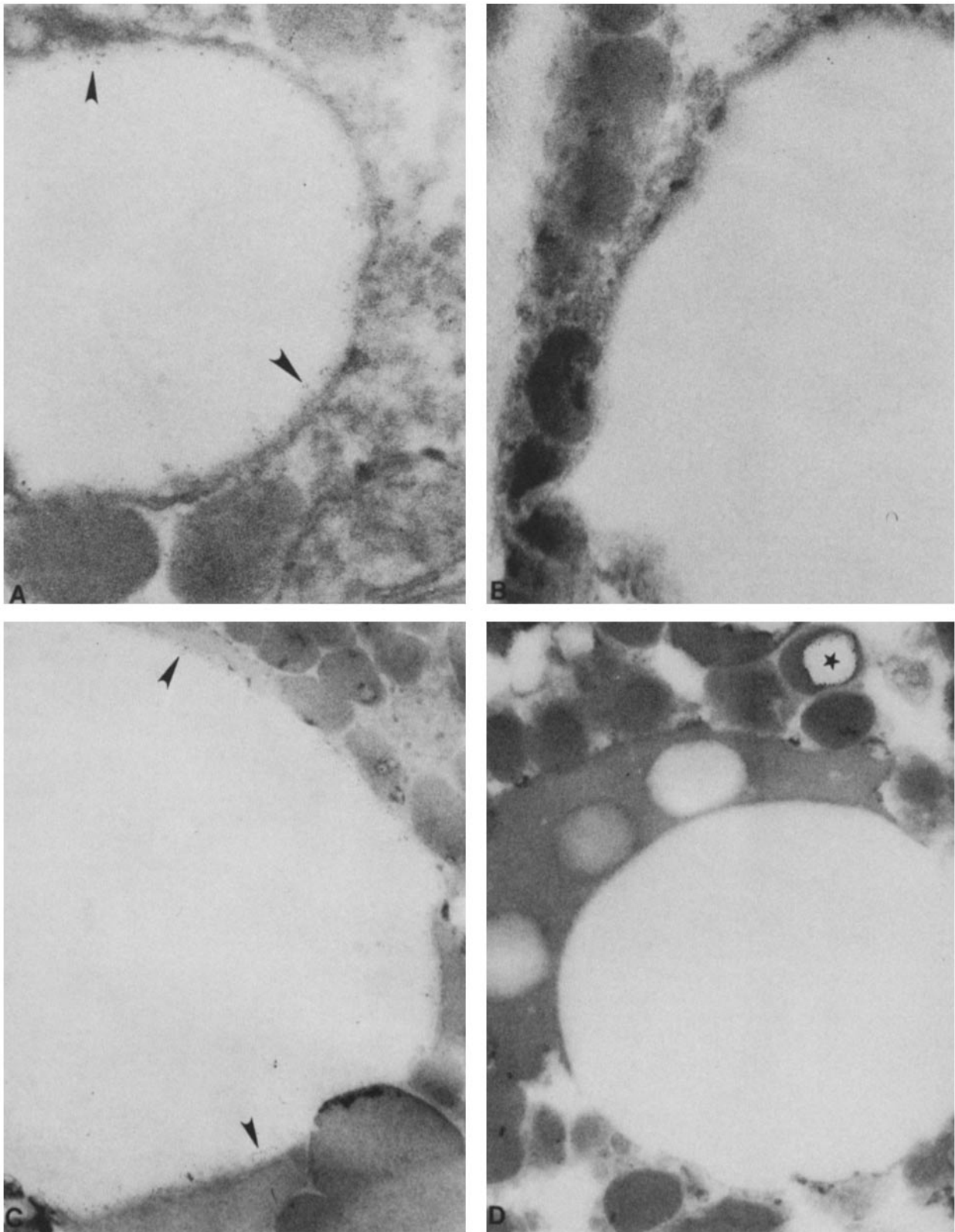


FIGURE 9 Electron micrographs of a radicle (A and B) and coleoptile (C and D) treated with AS (A, B, and C) or CS (D) followed by ferritin-labeled goat anti-rabbit immunoglobulin. Ferritin (arrowheads) is evident at the periphery of protein bodies located in cells at the surface of the radicle (A) but is absent from similar bodies in cells in the interior of the radicle (B). In the coleoptile (C), protein bodies also display reaction product at the periphery; ferritin is absent from similar cells treated with CS (D). The electron-opaque particles in D (star) do not represent ferritin; they are found in sections that were neither incubated with antibody nor poststained (not shown). Sections were poststained with uranyl acetate. A: $\times 100,000$. B: $\times 66,000$. C and D: $\times 43,000$.

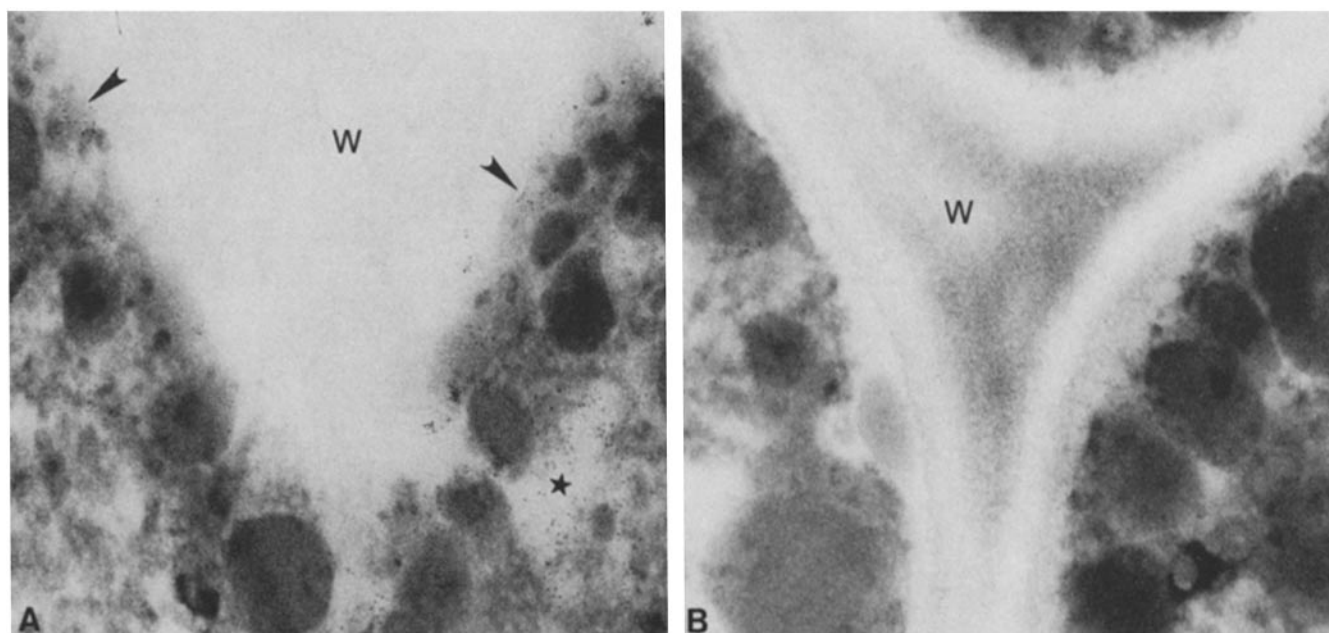


FIGURE 10 Electron micrograph of cells treated with AS (A) or CS (B) followed by ferritin-labeled goat anti-rabbit immunoglobulin. The cells were located at the periphery of the radicle, and the plane of section passes through the cell wall (W). Ferritin (arrowheads) is evident at the periphery of the cell adjacent to the cell wall and in electron-lucent areas of the cytoplasm (star). CS-treated cells display no reaction product (B). Sections were poststained in uranyl acetate. A: $\times 71,000$. B: $\times 67,000$.

least some of the lectin (28, 29) suggests a role in myoblast fusion, the function of the lectin in muscle development remains unclear (11, 18, 21). A lectin similar or identical to the muscle lectin is found in various embryonic (20) and adult (4, 7) tissues. The lectin is limited to particular regions in these tissues (4), including the extracellular space between pancreatic acini, secretory granules of goblet cells, and the area lining hepatic sinusoids. Thus, numerous lectins, including WGA, are found only in particular cell types, and often at cell surfaces or within cells at the periphery of certain organs. The localization patterns suggest that a common characteristic of these diverse proteins is a site of action that includes the extracellular environment.

Along with certain legume lectins, WGA is stable at elevated temperatures (up to 60°C ; 27) or in the presence of equimolar concentrations of numerous proteases (K. Keegstra, R. Wiedenhoft, and E. Sohlberg, unpublished results). Thus, both the wheat and legume lectins possess characteristics which would allow them to maintain their activity under harsh conditions, whether it be the digestive tract of a seed predator or the surface of a root. The low level of WGA in wheat grains as compared to the large amount of lectin present in numerous legume seeds argues against a role for WGA in the defense against seed predators (17). However, the fungicidal properties of purified WGA (24) coupled with the highly specific localization pattern we report here indicate that WGA may serve to impede fungal growth. Both during germination and while adventitious roots emerge from the shoot, openings form on the surface of the plant that could serve as routes of entry for pathogens. WGA is localized precisely at these potential infection sites.

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