# STUDIES OF FINE STRUCTURAL AND BIOCHEMICAL CHANGES IN COTYLEDONS OF GERMINATING SOYBEANS*

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[*Manuscript received April* 3, 1967]

*Summary*

Ultrastructural changes have been observed in the soybean cotyledon during early germination and subsequent seedling growth. Concurrently measurements were made of fresh and dry weight, chlorophyll and protein content, and porphyrin and protein synthesis.

For the first 2 days no major changes occurred other than swelling due to imbibition. The cells were tightly filled with protein bodies and lipid droplets. Incorporation of [$^{14}$C]glycine into protein was active but there was no porphyrin synthesis from δ-aminolaevulic acid.

From 2–10 days cotyledons expanded and became green and leaf-like: protein bodies expanded and coalesced to form the cell vacuole which became electron-optically empty, and lipid droplets disappeared from the cells which became highly vacuolated and rich in well-developed plastids. During this period the major loss in protein content and dry weight occurred. Protein and porphyrin synthesis was rapid.

After 10 days degenerative changes were seen in progressively more cells of the cotyledon: the tonoplast was broken down, the plastid membranes disrupted, mitochondria lost their substructure, and the nucleolus disappeared. The nuclear membrane and stroma ultimately degenerated and the cytoplasmic content of the cells was lost, leaving only isolated membranes which could not be clearly attributed to any single organelle. During this period protein synthesis became minimal and porphyrin synthesis could not be detected. The chlorophyll and protein content of the cotyledon was greatly reduced and there was a sharp fall in fresh weight.

## I. Introduction

In the study of seed germination, particular attention has been given to economically important seeds. Of these the soybean is of interest as its cotyledon shows three developmental phases during early germination and growth of the soybean plant.

Initially the cotyledon is the primary storage organ of the seed, which is rich in protein and fat [31 and 22% by weight respectively (Earle and Jones 1962)]. During seedling establishment levels of these compounds in the cotyledon diminish (Van Ohlen 1931). Then on about the third day of germination the cotyledons, which in the dormant seed lack chlorophyll, green rapidly and expand to become leaf-like

* Project supported by United States Department of Agriculture Grant under P.L. 480 to one of the authors (S.K.).

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and photosynthetic (Abrahamsen and Mayer 1966). Eventually the cotyledons become yellow, lose turgor, and fall from the plant.

Since the advent of electron microscopy the ultrastructural changes in certain seeds during and after germination have been studied in yucca (Horner and Arnott 1966), peanut (Bagley *et al.* 1963), lima bean (Klein and Ben-Shaul 1966), and in the common garden pea (Bain and Mercer 1966) and bean (Opik 1965), but none of these species undergo the full sequence of changes observed in soybean. The studies reported here trace changes in fine structure of the soybean cotyledon as it develops from a dormant achlorophyllous storage organ to a photosynthetic leaf and thence proceeds to its eventual senescence. Fresh and dry weight and chlorophyll and protein contents have been recorded and the capacity of the tissue to synthesize protein and porphyrins was determined.

## II. Materials and Methods

### (*a*) *Plant Materials*

Seeds of *Glycine max* (Ottowa Mandarin) were soaked in saturated sodium hypochlorite for 10 min, rinsed, and germinated on moist filter paper in an illuminated incubator at 26°C. After 3 days cotyledons were faintly green. At 4 days seedlings were transferred to the greenhouse and planted in a mixture of vermiculite and sand which had been saturated with Hoagland's nutrient solution and allowed to drain. Cotyledons were sampled from the dry seeds and from the germinating seeds and seedlings at 2–3-day intervals for 30 days. Similar material was also used in the photosynthetic and dark $^{14}CO_2$ fixation studies reported elsewhere (Abrahamsen and Mayer 1967). Staggered planting permitted harvesting of all ages on the same day. Fresh weight was recorded immediately and dry weight after 48 hr at 95°C.

### (*b*) *Chlorophyll and Protein Content*

Cotyledons were ground in 80% acetone and extracted three times in the same solvent until pigment-free. Extracts were combined and chlorophylls *a* and *b* calculated from optical densities at 645 and 662 mμ (Smith and Benitez 1955); such calculations assume that chlorophylls *a* and *b* are the only pigments in the extract absorbing at these wavelengths.

Protein was extracted by the method of Racusen and Foote (1960) and estimated by the biuret reaction using a standard curve obtained with bovine serum albumen (Sigma).

### (*c*) *Incorporation of δ-Aminolaevulic acid into Porphyrins*

δ-Aminolaevulic acid is the immediate precursor of porphobilinogen (Shemin and Russel 1953) and therefore a substrate for porphyrin formation (Bogorad and Granick 1953). Endogenous chlorophyll has a low fluorescence yield but porphyrins formed in response to δ-aminolaevulic acid give a characteristic red fluorescence in long-wave ultraviolet light. Cotyledons were cut transversely into slices about 1 mm thick and incubated in Petri dishes at 26°C in darkness on filter paper which had been moistened with 5 ml of 0·01M δ-aminolaevulic acid for 10 hr. The intensity of red

fluorescence in ultraviolet light was compared with that of controls incubated in water using an arbitrary four-point scale.

### (*d*) *Protein Synthesis*

Cotyledons were sliced as before and incubated with 1 $\mu$c of [$^{14}$C]glycine in 0·1 mM glycine in white light of 800 f.c. for 4 hr at room temperature. Incubations were terminated by plunging the cotyledon slices into boiling 80% acetone. Samples were ground in glass homogenizers and chlorophyll extracted by repeated centrifugation and resuspension in 80% acetone. The pigment-free pellet was resuspended three times in 5 ml 1N $HClO_4$ for 15 min in a boiling water-bath to remove nucleic acids. Protein was extracted by resuspension in 1N NaOH in the same way. The protein solution was chilled, and excess cold trichloroacetic acid added to a final concentration of 5%. After centrifugation the protein was dissolved in 0·1N NaOH and reprecipitated with trichloroacetic acid. This precipitate was deposited on a Millipore filter which was then washed with cold 5% trichloroacetic acid, dried, and assayed in a Packard Tri-Carb liquid scintillation counter. The sample was fractionated so that the protein deposited did not exceed 5 mg; above this level self-absorption errors were introduced.

### (*e*) *Electron Microscopy*

An initial survey of cotyledon anatomy from hand-cut sections in the light microscope (Plate 1, Fig. 1) indicated that the two layers of palisade cells near the adaxial epidermis provided a reasonably uniform tissue for electron-microscopic investigation. Blocks of tissue were taken from the middle of the adaxial surface of the cotyledon taking care to include the epidermis (as a point of reference in subsequent fine structure studies) and the palisade and part of the mesophyll.

Tissue was fixed in 6% glutaraldehyde (4 hr) and post-fixed with 2% $OsO_4$ (1 hr) (both at 0–2°C and phosphate buffered at pH 7·6) or in 2% $KMnO_4$ (1 hr) and dehydrated in graded alcohols before embedding in Epon. Ultra-thin sections were cut with glass knives and examined in an RCA EMU 3-G electron microscope. Glutaraldehyde-fixed specimens were after-stained with uranyl acetate or lead citrate.

## III. Results and Discussion

### (*a*) *Gross Changes during Germination*

Two days after the initiation of germination the seed coat was split and the radicle had emerged. At 3 days the cotyledons were pale green and at 7 days they were dark green and spreading away from the primary leaf which remained folded. At 10 days the cotyledons reached their maximum expansion and at 14 days their abaxial surface was pale green and at this stage the first trifoliate leaf elongated. At 17 days the cotyledon was flaccid, its upper surface pale green, and lower surface yellow; the first trifoliate leaf had expanded. After 21 days the cotyledon was wrinkled and only faintly green or in some cases yellow.

An examination of hand-cut cotyledon sections at low power in the light microscope showed that greening occurred initially in the adaxial palisade and was

followed by greening of the cells adjacent to the abaxial epidermis and lastly in the mesophyll. Chlorophyll disappeared in the reverse order: first from the mesophyll which became pigment-free and then from the cells close to the abaxial epidermis, and finally from those close to the adaxial epidermis which became yellow.

Fresh weight of the cotyledons (Fig. 1) rose rapidly during imbibition and, after a 2-day lag, continued to increase. This secondary gain occurred during a period in which the cotyledon was expanding into a leaf-like photosynthetic organ but while its dry weight was decreasing. After 10 days the fresh weight dropped rapidly and dry weight remained fairly constant. Changes in fresh weight corresponded closely to the changes in respiration rate which also reached a peak at 10 days (Abrahamsen and Mayer 1967).

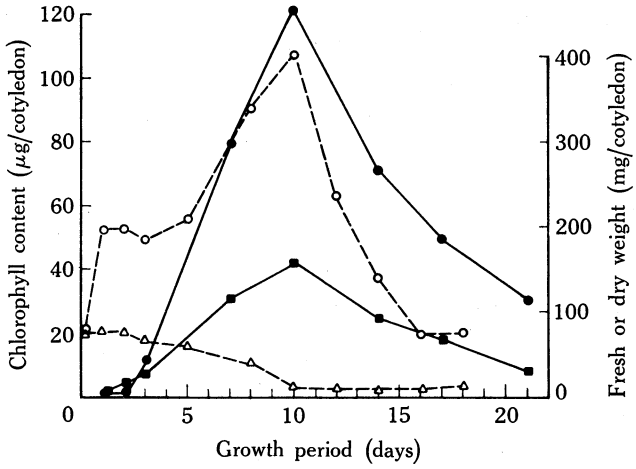


Fig. 1.—Fresh weight (○), dry weight (△), and chlorophyll *a* and *b* content (●, ■ respectively) of cotyledons from soybean seeds and seedlings at various times after the initiation of germination.

### (*b*) *Biochemical Changes during Germination*

(i) *Chlorophyll Content.*—Chlorophyll content (Fig. 1) increased rapidly after 2 days and reached a peak for both chlorophyll *a* and chlorophyll *b* at 10 days, and corresponded with the changes in total chlorophyll content and photosynthetic activity observed by Abrahamsen and Mayer (1967). The higher concentration of chlorophyll *b* during the initial stages of greening differs from the usual pattern of chlorophyll accumulation. This result was reproducible but calculations were based on the optical density of the acetone extract. The pigments were not isolated. Both chlorophylls accumulate rapidly only when fresh weight is increasing. Loss of chlorophyll is closely correlated with the dehydration of the tissue during a period when dry weight is relatively constant.

(ii) *Porphyrin Biosynthesis.*—Incorporation of δ-aminolaevulic acid into chlorophyll (Marsh, Evans, and Matrone 1963) or other porphyrins (Carell and Price

1965) has been used to provide a measure of the capacity of a tissue for porphyrin biosynthesis. An estimate of porphyrin biosynthesis in soybean cotyledons, as indicated by red fluorescence of tissue slices in long-wave ultraviolet light after incubation for 10 hr in the dark, is given in the following tabulation:

| Treatment | Days after Germination | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 5 | 7 | 10 | 14 | 17 |
| δ-Aminolaevulic acid (0·01M) | – | – | ++ | +++ | +++ | + | – | – |
| Water | – | – | – | – | – | – | – | – |

Tissue from cotyledons 2 days after imbibition showed no detectable red fluorescence after incubation for 10 hr with δ-aminolaevulic acid. In cotyledon slices from plants 3–7 days old fluorescence was more intense. At 10 days only a faint red fluorescence was detected and none was detected at 14 and 17 days. At the intensity provided by the ultraviolet source used, fluorescence of endogenous chlorophylls could not be observed. The capacity for porphyrin synthesis only from day 3 to day 10 corresponds with the period of chlorophyll accumulation (Fig. 1).

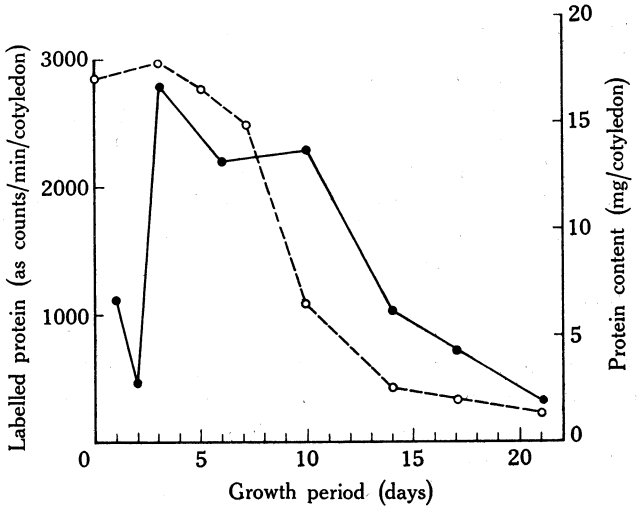


Fig. 2.—Total protein (○) and incorporation of [$^{14}$C]glycine into protein (●) in cotyledons of soybean seeds and seedlings.

(iii) *Protein Content and Biosynthesis.*—Protein content (Fig. 2) fell sharply after 5 days. Two-thirds of the protein content was lost while fresh weight, chlorophyll content, and respiration rate (Abrahamsen and Mayer 1967) increased rapidly. This loss was apparently due to the transfer of storage protein to other parts of the seedling. Protein synthesis, as indicated by the incorporation of [$^{14}$C]glycine into material which could be precipitated with trichloroacetic acid, was high at day 1, dropped at day 2 (in each of four experiments), and then rose sharply, reaching a peak for cotyledon slices from plants 3–10 days old. Active protein and chlorophyll synthesis

occurred during a period of net protein loss. This loss did not affect plastid formation. Chlorophyll accumulation occurs only when protein synthesis and porphyrin synthesis is rapid (Treffry 1965).

### (*c*) *Electron Microscopy*

In attempting to compare the biochemical characteristics of an organ and cellular fine structure it is emphasized that the former concern the entire organ and the latter are restricted to a small portion of it.

We must also point out, as did Shaw and Manocha (1965), that degenerating organelles are more easily damaged than normal ones during preparation for electron microscopy. However, tissues were always handled in the same way and at the same time. Differences in the fine structure of cells and organelles were reproducibly related to the age of the cotyledon and are either "real" or indicate that such tissues are affected differently by the preparative procedures employed (which were the same for cotyledons of various ages). Cell to cell variation in the response of the tissue particularly during the later stages of senescence tends to obscure the sequence of the terminal changes.

### (*d*) *General Changes in Appearance of the Cells*

Cotyledon fragments from dry seeds were placed directly in the fixative; this material was regarded as representing the condition in the resting seed or at least in the earliest stage of germination. Fixatives were in aqueous solution and some imbibition occurred; but in no treatments did this continue for longer than 2 hr before dehydration in the alcohol series commenced. Cells of the palisade were for the most part tightly packed with few intercellular spaces. The cell walls (Plate 1, Fig. 2) were relatively thick. There was no central vacuole but there were several membrane-bounded areas with a homogeneous or slightly granular content; these have the same appearance and staining characteristics as the protein bodies found in peanut (Bagley *et al.* 1963). The cytoplasm was densely packed with lipid droplets compressed into angular shapes (Plate 1, Fig. 2). The few plastids observed (Plate 1, Fig. 2) contained a starch-like granule but no internal membranes. Cells were very rich in ribosomes but no reticulum was observed except in cells of the epidermis which at all stages had a fine structure typical of palisade cells in cotyledons 2 or 3 days older.

At day 1 the cells had expanded, cell walls were straighter and thinner, and the protein bodies appeared to enlarge and some to coalesce (Plate 2, Fig. 1). Lipid droplets were less densely packed. Double membranes were seen inside some plastids (Plate 1, Fig. 3). At 3 days lipid bodies were more scattered and the endoplasmic reticulum could now be observed (Plate 2, Fig. 2). Protein bodies in some cells had joined to form a single central vacuole with granular contents. In the peanut (Bagley *et al.* 1963) and the garden pea (Bain and Mercer 1966), protein bodies lose their granular content during germination and break down. Preliminary studies with developing soybean cotyledons indicate that in this case the protein bodies not only give rise to the cell vacuole during germination but are themselves formed by partitioning of the vacuole during the dehydration phase of seed ripening. At 5 days vacuolation was

completed and the cytoplasm and organelles were restricted to the cell periphery. At 7 days vacuolar content remained stainable. At 10 days, when the period of most rapid loss in protein content (Fig. 2) had passed, the vacuole appeared "empty" after both permanganate and glutaraldehyde–osmium fixation. In some cells the tonoplast appeared disrupted and the nucleus highly granular. Large intercellular spaces often exceeded the dimensions of the cells. In subsequent days the cytoplasm was greatly reduced and organelles appeared to break down (Plate 3, Figs. 1 and 2). The protoplast was no longer confined to the cell periphery (Plate 3, Fig. 3) but formed clumps (Plate 5, Fig. 1) or bands (Plate 4, Fig. 2) in the cell lumen. More frequently cytoplasmic stroma had completely disappeared and the cell lumens contained only degenerating organelles (Plate 4, Fig. 2; Plate 5, Figs. 1 and 2; Plate 6, Fig. 2) and disorganized membranes (Plate 6, Fig. 3).

### (*e*) *Changes in Cell Organelles*

(i) *Nucleus.*—The nucleus appeared unchanged for the first 7 days after germination. The nucleolus was prominent and dark granules (the chromatin) were concentrated at the periphery of the nucleus which was often cup-shaped (Plate 1, Fig. 3). In the nucleus of some cells at 7 days the chromatin was less apparent or diffusely spread in the nuclear stroma. At later stages the nuclear membrane became less distinct (Plate 3, Fig. 3) and the nuclear stroma vacuolated (Plate 5, Fig. 1; Plate 6, Fig. 2).

(ii) *Plastids.*—In the resting seed, plastids lacked internal membranes and contained starch-like granules and an undifferentiated stroma (Plate 1, Fig. 2). These granules differ from the starch grains observed in pea (Bain and Mercer 1966) which are not surrounded by a plastid membrane. Some development of internal membrane had occurred 3 days after imbibition when the cotyledons are faintly green (Plate 2, Fig. 2). Elaboration of grana continued until day 7 (Plate 2, Fig. 3). During this period chlorophyll accumulated rapidly and coincided with the development of internal membranes in the plastids (Virgin, Kahn, and von Wettstein 1963). At 10 days some plastids, particularly in permanganate-fixed preparations (Plate 3, Figs. 1 and 2), were greatly swollen, their limiting membrane ruptured, and thylakoids expanded. In glutaraldehyde-fixed tissue (Plate 4, Fig. 1) plastid stroma was highly granular. The outer thylakoids in the grana appear to swell and their interior is electron-optically empty. It would seem that plastid degeneration had already commenced when the peak in fresh weight, chlorophyll content, and respiratory and photosynthetic activity was reached. In cells of 14-day-old cotyledons the swollen plastids contained few very large grana (Plate 4, Fig. 2). In some cells grana remained intact even at 21 days (Plate 5, Fig. 1) but the stroma was highly granular (Plate 5, Fig. 2) with many lipid droplets (Plate 5, Fig. 1; Plate 6, Fig. 2); these probably contained the residual carotenoids colouring the tissue.

The manner of plastid breakdown was similar to that observed in detached wheat leaves maintained on distilled water in the light (Shaw and Manocha 1965) and also in certain nutrient deficiencies (Thomson and Weier 1962; Vesk, Possingham, and Mercer 1966). This swelling and disruption may be a response to fixation after

membrane degeneration caused by almost any factor interrupting normal metabolism and may not represent the condition of the organelles *in vivo*. In most senescent cells cytoplasm was entirely lacking and plastids are represented by residual membranes and small starch grains (Plate 6, Fig. 3). In the ripening cotyledon, plastid breakdown also occurs but preliminary results indicate that the mechanisms involved differ; plastids in the maturing cotyledon become charged with starch and the plastid membrane remains intact.

(iii) *Mitochondria.*—Mitochondria were not observed in resting cells (cf. Opik 1965) perhaps due to the dense array of lipid droplets obscuring other structures. They are frequently seen after 3 days. Mitochondria often appear to be included in plastids (Plate 3, Fig. 3; Plate 4, Fig. 1). In such cases closer examination shows a thin band of cytoplasm and a plastid membrane surrounding the mitochondria. In the cells where the plastids are disrupted vesicles are seen which lack internal structure. These vesicles are tentatively identified as degenerate mitochondria (Plate 3, Fig. 1; Plate 4, Fig. 1).

(iv) *Reticulum and Ribosomes.*—Ribosomes were frequent in resting cells but reticulum with ribosomes attached was not seen (in palisade cells) before 3 days (Plate 2, Fig. 2). Reticulum and ribosomes were most prominent at 7 days (Plate 2, Fig. 3) and were still apparent in residual areas of cytoplasm in some cells even at 32 days (Plate 6, Fig. 1). Such isolated concentrations of ribosomes may have accounted for the amino acid incorporation which is still detectable in senescent tissue. In general, reticulum was difficult to identify during the final stages of senescence but may be represented by the vesicles or isolated membranes observed in Plate 6, Figure 3.

(v) *Lipid Bodies.*—Lipid droplets are tightly packed in the cytoplasm of resting cells (Plate 5, Fig. 1). As the cells expand they are distributed mainly along cell walls or around vacuoles. "Holes" in these bodies (Plate 2, Fig. 2) may be due to utilization of the lipid but are more probably a fixation artefact as their staining characteristics seem to be highly dependent on the extent to which the tissue is rinsed between the glutaraldehyde and osmic acid fixations. Lipid droplets were not seen in the cytoplasm after 10 days but later became very frequent in the plastids (Plate 5, Fig. 1), and sometimes occupied almost the entire plastid volume (Plate 6, Fig. 2).

During the germination and establishment of the soybean plant the cotyledons function as a nutritive source. Their initially high content of dry matter, lipid, and protein is lost, presumably to the axis. Superimposed on this function is the development, transient maturity, and senescence of the cotyledon as a photosynthetic organ. The ultrastructural features of both these roles can be closely correlated with the biochemical and physiological characteristics of the cotyledons during this sequence of events.

## IV. Acknowledgments

The authors' thanks are due to Miss Amyra Yevzori and Mr. E. Chatab for their assistance in the preparation of specimens and electron microscopy.

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## Explanation of Plates 1–6

Light-micrograph (Plate 1, Fig. 1) and electron-micrographs of transverse sections of soybean cotyledons from seeds and seedlings at various times after germination. The light-micrograph is of a hand-cut section of fresh material. The remaining electron-micrographs are of tissue fixed in potassium permanganate only, or fixed in glutaraldehyde (G), post-fixed with osmic acid (O), and after-stained with uranyl acetate (U) or lead citrate (P)

### Plate 1

Fig. 1.—Hand-cut transverse median section of soybean cotyledon after 1 day of imbibition. Note area representative of tissue taken for electron-microscopic examination. The area includes adaxial epidermis and adjacent cells. ×145.

Fig. 2.—Portion of palisade cell from tissue fixed without previous imbibition. Protein bodies (*B*) have a homogeneous content. Plastids contain starch grains (*S*) but have no internal membranes. Lipid droplets (*L*) occupy the remaining cell volume and in places are tightly packed and angular (arrows). Cell walls (*W*) are relatively thick. G, O, and U.

Fig. 3.—One day after the initiation of germination showing nucleus (*N*) with nucleolus (*n*) and chromatin (*C*). Protein bodies (*B*) have a granular content and a more clearly defined limiting membrane. Plastids contain internal membranes (arrow). Lipid bodies are less densely packed and the cytoplasm surrounding them is rich in ribosomes (shown more clearly at higher magnification). Cells have expanded and the cell walls (*W*) are thinner than in the previous figure. G, O, and U.

## PLATE 2

Fig. 1.—Same stage as Plate 1, Figure 3, but fixed with potassium permanganate. Protein bodies (*B*) lack content, so also do the lipid droplets (*L*).

Fig. 2.—Portion of palisade cell after 3 days. Plastid contains starch (*S*) and membranes (arrow). Lipid droplets (*L*) have electron-optically empty areas which may be artefacts. Mitochondria (*m*) and endoplasmic reticulum (*R*) with ribosomes are observed. The cytoplasmic matrix is more apparent and heavily charged with ribosomes. G, O, and U.

Fig. 3.—After 7 days the protein bodies have coalesced to form a central vacuole (*V*) whose contents are more sparsely stained. Plastids contain many grana but little starch (*S*). Lipid droplets (*L*) are less frequent. Mitochondria (*m*) and endoplasmic reticulum (*R*) are well developed. *W*, cell wall. G, O, and P.

## PLATE 3

Figs. 1 and 2.—Portion of cells in cotyledons of 10-day-old seedlings in which the tonoplast (*T*) has broken down and the cytoplasm degenerated. The mitochondria (*m*) lack internal membranes and the plastids (*P*) contain many vesicles derived from disrupted grana. The plastid membrane (arrows) is broken. In some cases (Fig. 2) the plastid is ruptured, but the grana is intact. *W*, cell wall. Potassium permanganate fixation only.

Fig. 3.—At 17 days cells are highly vacuolated and the vacuole (*V*) appears empty. The chromatin and membrane of the nucleus (*N*) is poorly defined. The internal structure of the plastids (*P*) is indistinct and they contain lipid droplets. The cytoplasm is reduced to a thin layer surrounding the organelles and is often withdrawn (arrows) from the cell wall (*W*). *S*, starch grain; *n*, nucleolus; *m*, mitochondria. G, O, and U.

## PLATE 4

Fig. 1.—Plastid in palisade cell of cotyledon from 17-day-old seedling. External thylakoids of some grana (*G*) are swollen and empty (arrows). Some mitochondria (*m*) have lost internal structure. The tonoplast (*T*) is beginning to break down. G, O, and U.

Fig. 2.—Large grana (*G*) in plastids of 14-day-old cotyledon. The plastid stroma contains vesicles and lipid droplets but large areas are unstained. *V*, vacuole. G, O, and U.

## PLATE 5

Fig. 1.—Portion of a cotyledon cell from a 21-day-old plant. The protoplast is restricted to an isolated "glob". The nuclear stroma (*N*) is vacuolated. The plastids (*P*) contain large lipid droplets (*L*). Myelin figures, vesicles, and reticulum can be identified in the residual cytoplasm. *Lu*, lumen. G, O, and U.

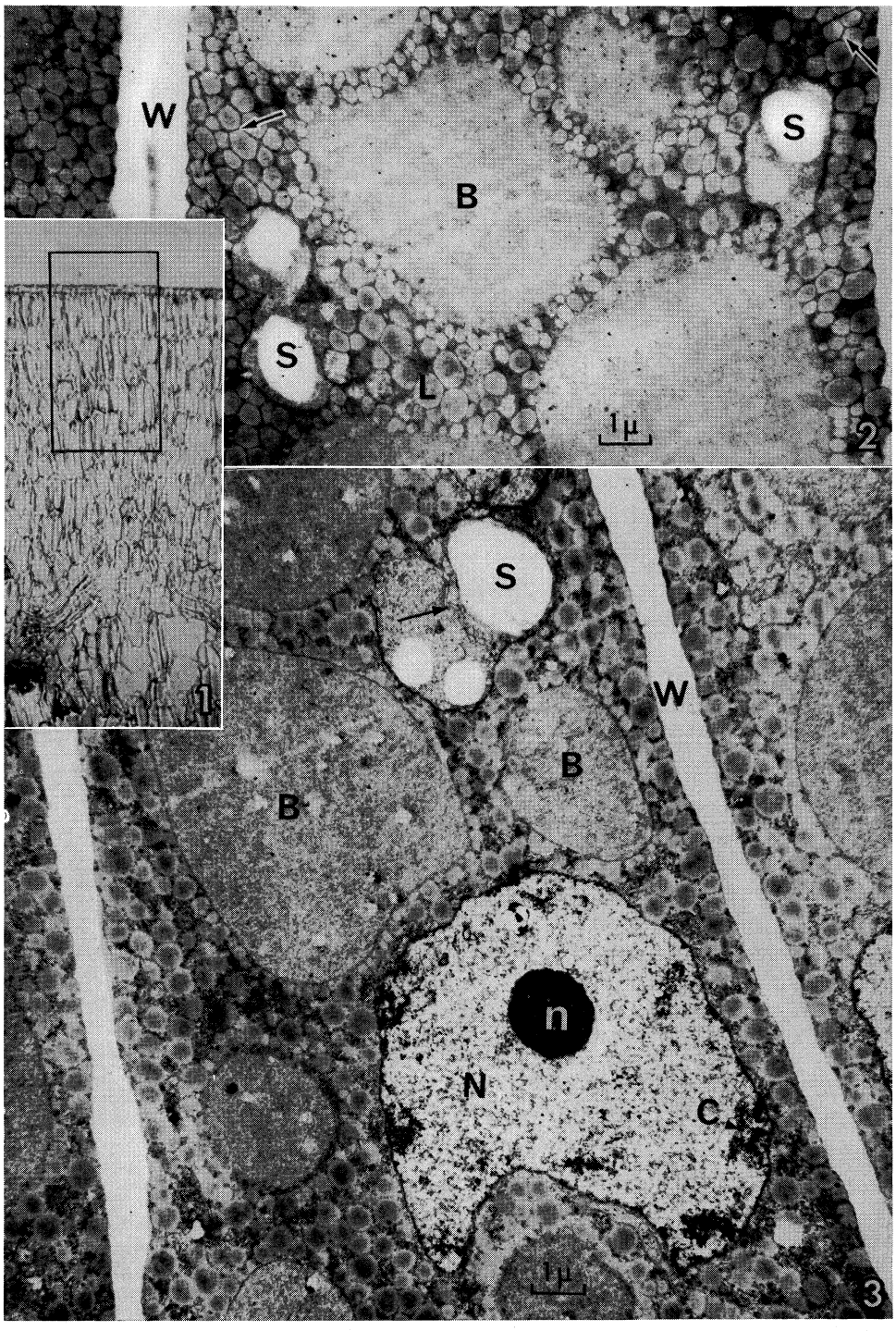
Fig. 2.—Degenerating nucleus (*N*) and plastids (*P*) scattered in the cell lumen (*Lu*). 14-day-old cotyledon. G, O, and U.

## PLATE 6

Figs. 1 and 2.—Portions of cells from an atypical cotyledon remaining attached to a 32-day-old plant. Nucleus (*N*) may appear normal (Fig. 1) or vacuolated (Fig. 2) and plastids (*P*) contain grana or lipid droplets (*L*). Ribosomes are seen attached to reticulum (*R*, Fig. 1) or to vesicles (Fig. 2, arrow). The tonoplast (*T*) may appear intact (Fig. 1). The vacuole (*V*) is empty. *S*, starch grains. G, O, and U.

Fig. 3.—Lumen (*Lu*) of senescent cell containing starch grains (*S*) and residual membranes. 17-day-old cotyledon. G, O, and U.

COTYLEDONS OF GERMINATING SOYBEANS



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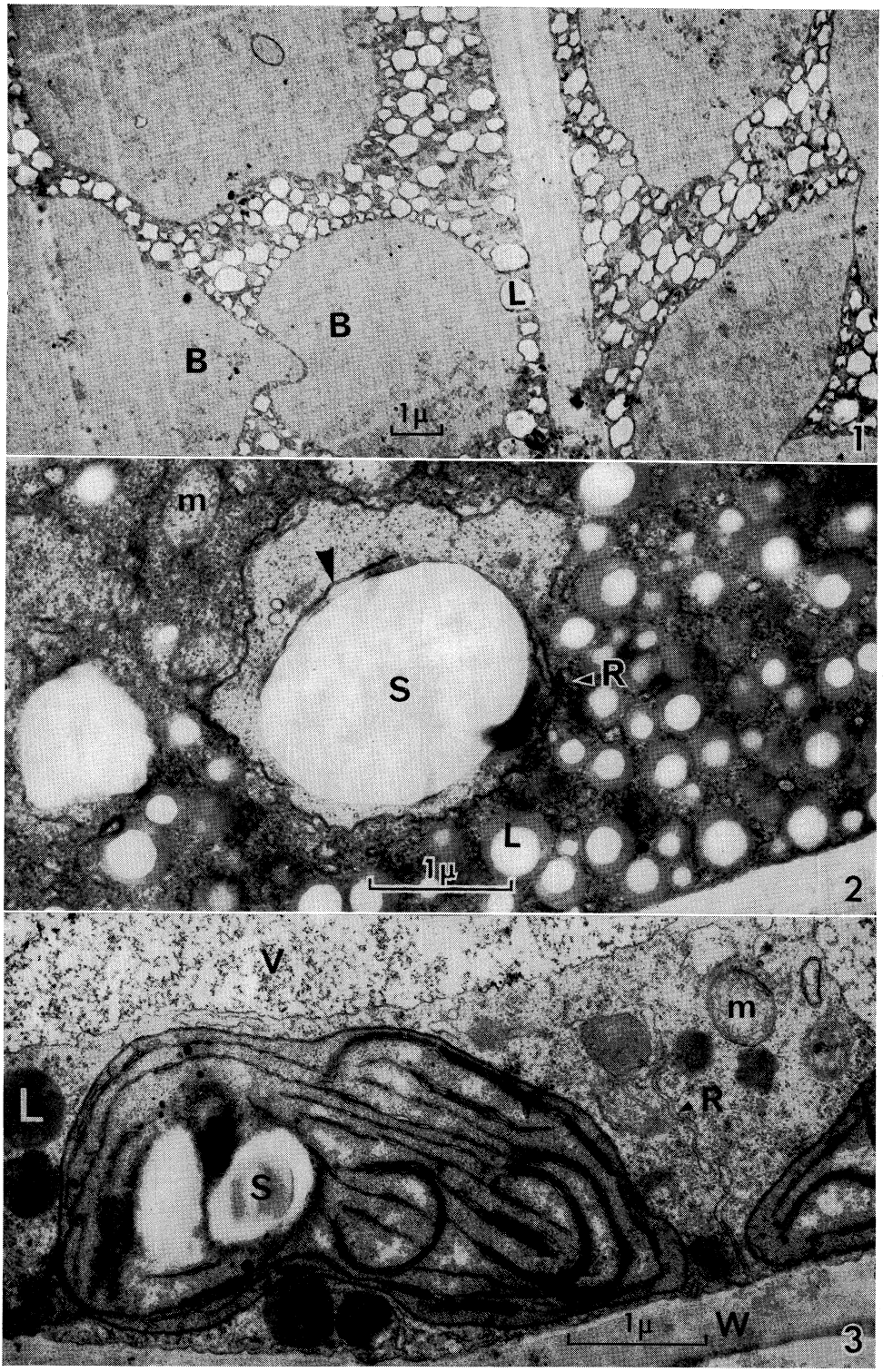

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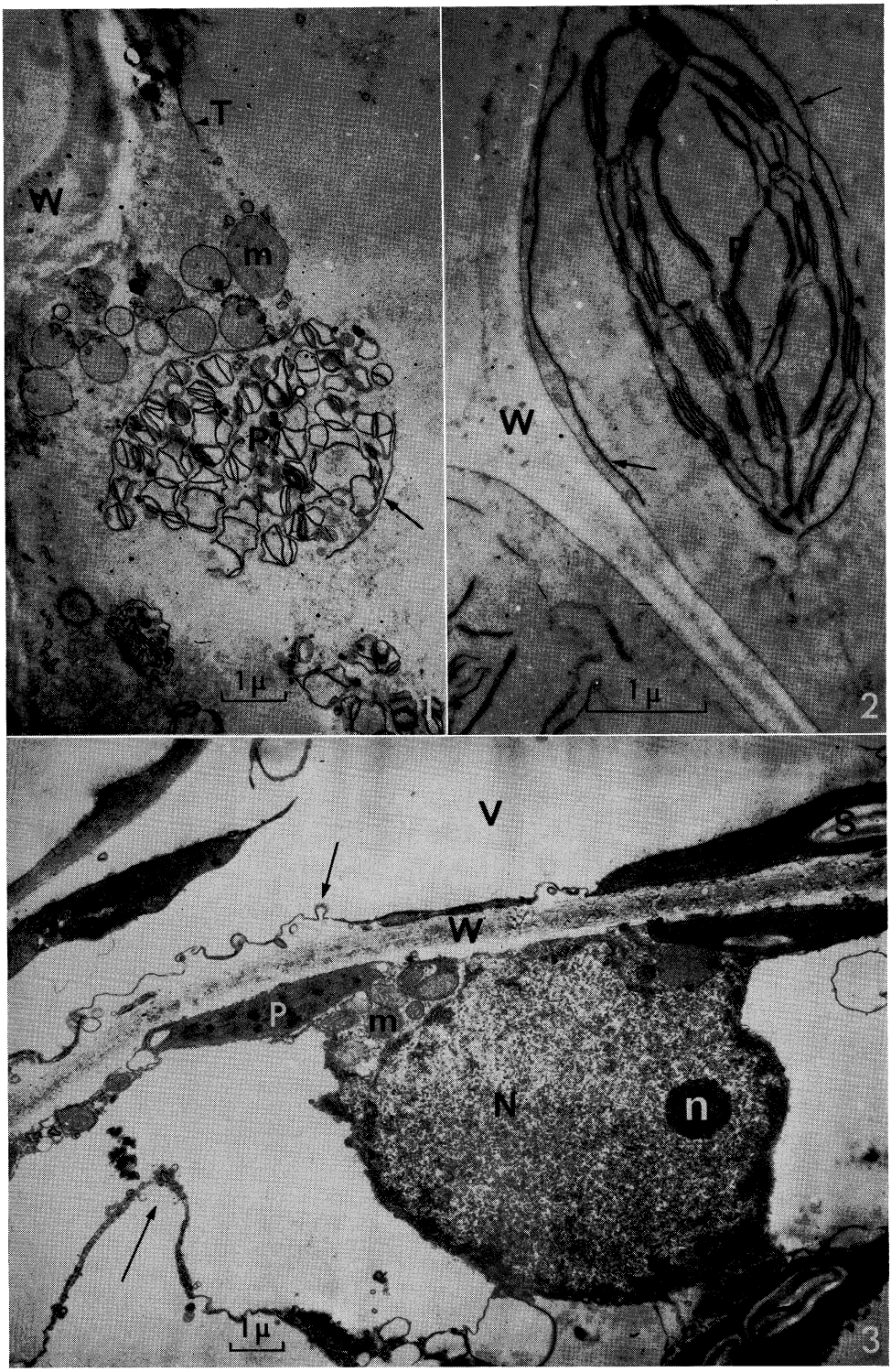

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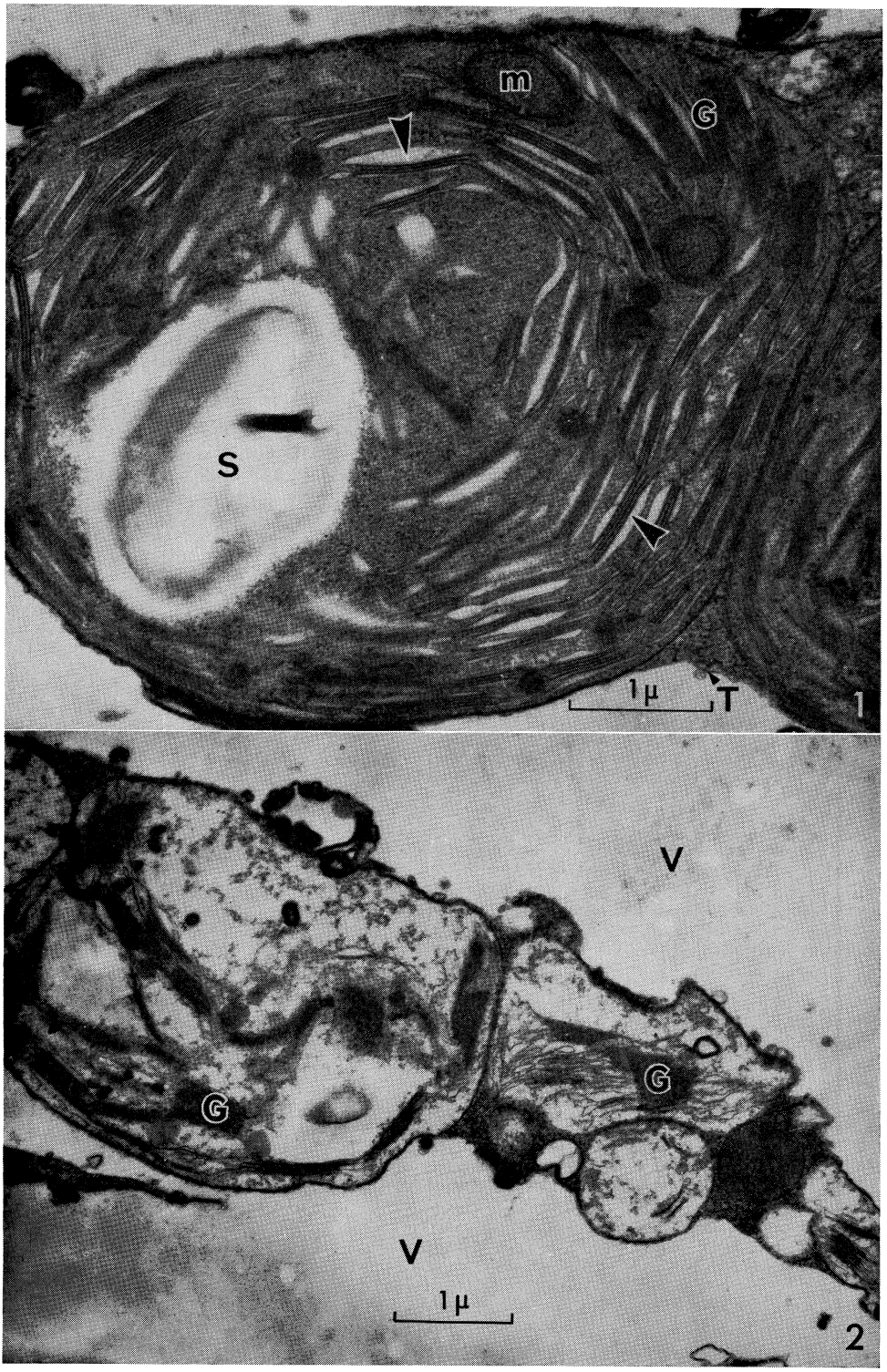
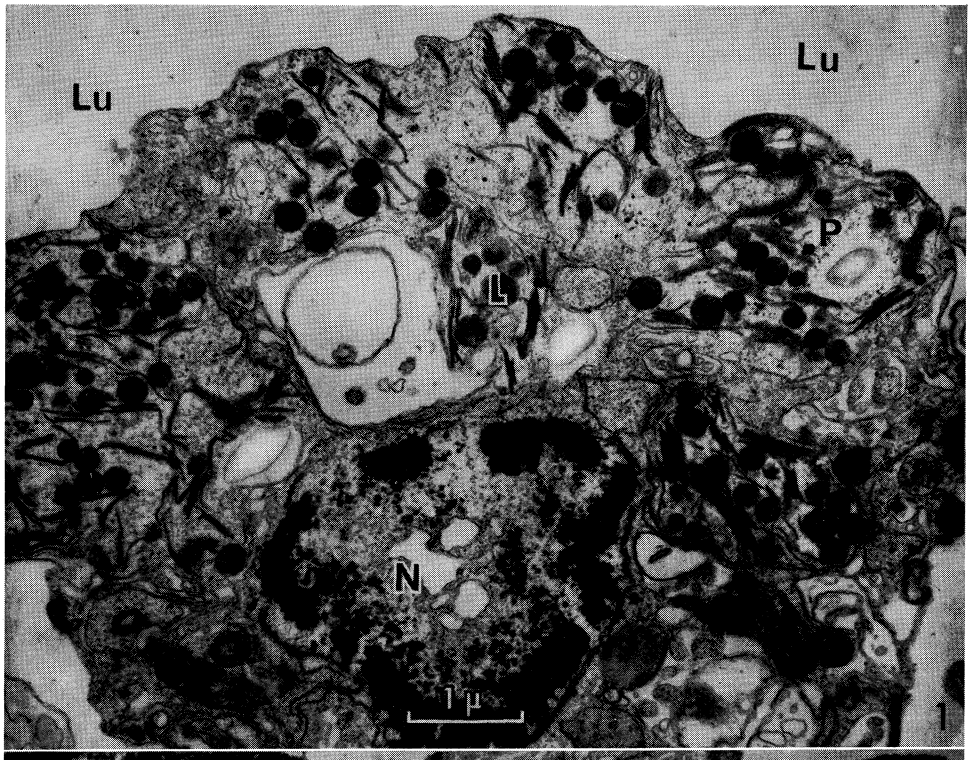
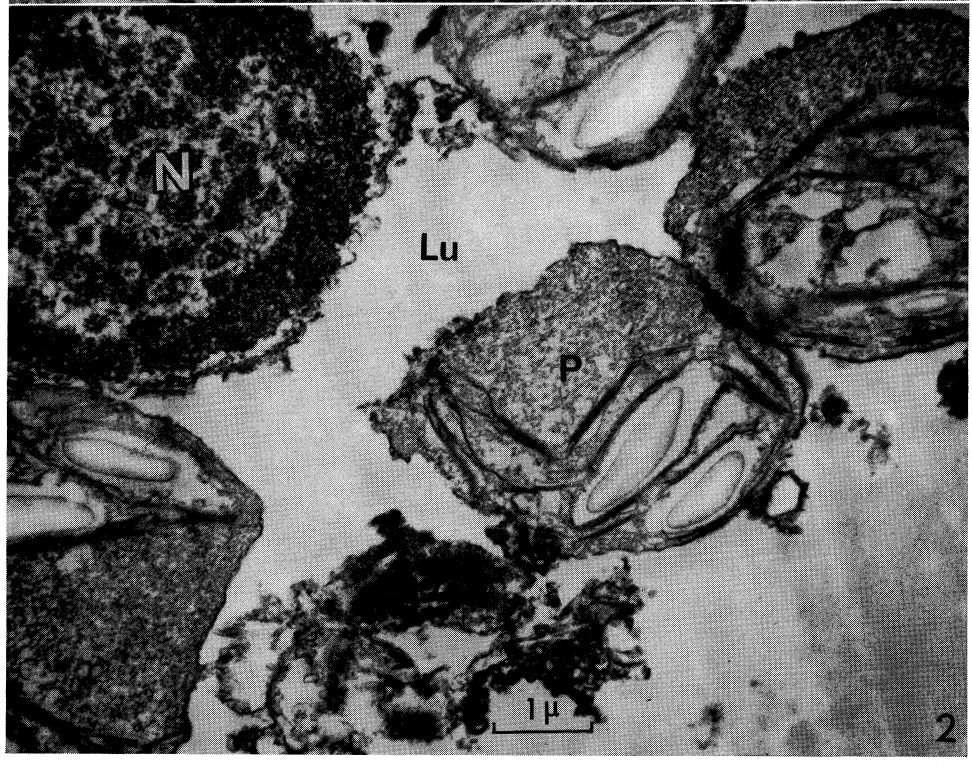


COTYLEDONS OF GERMINATING SOYBEANS



COTYLEDONS OF GERMINATING SOYBEANS