SUBCELLULAR ORGANIZATION OF THE COTYLEDONS IN GERMINATING SEEDS AND SEEDLINGS OF *PISUM SATIVUM* L.

By JOAN M. BAIN* and F. V. MERCER†

[*Manuscript received September* 6, 1965]

Summary

Ultrastructural changes observed in pea cotyledon cells during germination of the seed and establishment of the seedling have been related to parallel anatomical and physiological changes in the cotyledons, observations being made at daily intervals up to the 10th day, and less frequently up to the 22nd day from planting. The changes have been related to three distinct phases recognized in the development of the seedling.

Phase 1 in seedling development lasted 5 days, the seed germinating in the first 2 days and the radicle subsequently lengthening to 40 mm, mainly by cell elongation; the shoot system remained covered by the split testa. Starch and protein reserves were very conspicuous in the cotyledon cells. Starch grains were always in direct contact with the cytoplasm, but the protein material was at all times enclosed within a membrane. Breakdown of starch and protein reserves commenced as the tissue rehydrated but the reserves were not lost from the cotyledons. Only a small amount of subcellular structure could be resolved in the dormant cotyledon cells, but more became evident as metabolic activity increased following the breaking of dormancy. Increasing water content in the cotyledons was associated with increasing development of membrane systems in the cotyledon cells. Mitochondrial structure was fully formed 2 days after planting and was related to increasing respiration rate. A very extensive endoplasmic reticulum system formed in the cytoplasm during phase 1; this appeared to function as a temporary storage site for soluble reserves which were increasing. Nuclei enlarged and became very lobed; ribosomes were resolved in the cytoplasm.

Phase 2 lasted 3 days: the radicle grew to about 80 mm and developed secondary primordia; the shoot system expanded above ground level; and the leaves became green. Reserves began to move from the cotyledon. The ultrastructure pattern, built up during phase 1, began to break down. Disorganization was especially noticeable in the endoplasmic reticulum.

Phase 3 covered the remainder of seedling growth, the cotyledons beginning to deteriorate 10–12 days after planting. The greatest loss of storage reserves was during this phase. Disorganization of ultrastructure continued into phase 3, the cells then resembling those of senescent tissue. Deposits of fat increased in the cytoplasm with disorganization of membrane systems.

I. INTRODUCTION

Cotyledons of seeds during germination and the establishment of the seedling are useful material in which to follow the relationship of subcellular organization and cell function, since breaking of dormancy, recovery of metabolic activity, breakdown of storage reserves, and the loss of soluble products to the developing axis occur

* Division of Food Preservation, CSIRO, North Ryde, N.S.W.

† School of Biological Sciences, University of Sydney; present address: Macquarie University, Eastwood, N.S.W.

during these phases of growth. Cotyledons of *Pisum sativum* L. cv. Victory Freezer were used in the present investigation because the structural pattern of their cells in developing and ripening seeds has already been observed (Bain 1964; Bain and Mercer 1966).

This paper is mainly concerned with reporting ultrastructural changes observed in the pea cotyledons during the first 3 weeks after planting. These have been related to physiological and anatomical changes occurring at the same time in the cotyledons, fuller accounts of which have been given elsewhere (Bain 1964).

Considerable information on physiological and biochemical changes in germinating pea seeds is available in the literature (Malhotra 1933; Danielsson 1951; Young 1957*a*, 1957*b*; Spragg and Yemm 1959; Young and Varner 1959; Young *et al.* 1960; Varner, Balce, and Huang 1963; Varner and Schidlovsky 1963). A summary of seed germination is found in Crocker and Barton (1953), Toole *et al.* (1956), Koller *et al.* (1962), and Mayer and Poljakoff-Mayber (1963).

II. Materials and Methods

(*a*) *Source of Material*

Dried seeds of *Pisum sativum* cv. Victory Freezer (Canner's 75 or 99L) were planted 1 in. below the surface in fine glassworks sand during July, and grown over 3 weeks in a glasshouse without temperature control. This period covered the breaking of dormancy, germination (imbibition appeared complete and the radicle had broken the testa by the second day), and the establishment of the seedling.

Two treatments were used, the seeds being watered throughout the experiment either with distilled water or full culture solution (Hoagland and Arnon 1939). Increased growth was observed in the latter 1 week after planting, but as comparable physiological trends were observed throughout in the cotyledons under both treatments only the material subjected to distilled water will be discussed. In all, 110 seeds or seedlings were harvested daily up to the 10th day and at the 12th, 15th, and 22nd day from planting. Dormant seeds were used to obtain data for day 0.

(*b*) *Determinations at Each Sampling*

(i) *Fresh Weight, Dry Weight, and Moisture Content.*—One hundred seedlings were used in finding first their average fresh weight and then that of their dissected parts, viz. testa, two cotyledons, and axis. Dry weight (and moisture content) of the dissected parts was found after drying at 80°C or 24 hr. That of the seedling was then calculated. The fresh weight and dry weight of 100 embryos taken from dormant seeds was found to give data for day 0. These embryos were approximately 84% of the fresh weight and 92% of the dry weight of the seed.

(ii) *Nitrogen and Carbohydrate Content.*—The dried cotyledons from the 100 seedlings were finely ground and the level of total nitrogen, protein nitrogen, starch, total sugar, and reducing sugar was found per pair of cotyledons, using methods described previously (Bain and Mercer 1966). One hundred whole seeds were used to give day 0 values for nitrogen and carbohydrate, the embryo contributing the greater part of the content for the ripened seed—98% of the total nitrogen, 97% of the protein nitrogen, 98% of the starch, and 98% of the total sugar (Bain 1964).

(iii) *Respiration Rate.*—Oxygen uptake by dormant seeds and by cotyledons removed from seedlings after 1, 2, 3, 4, 5, 7, 9, and 15 days' planting was determined by the Warburg manometric technique. Five dormant seeds or two cotyledons from five seedlings at each sampling were placed in Warburg vessels at 25°C. Measurements expressed as microlitres of oxygen per 5 min per gram fresh weight and then per pair of cotyledons were found for all samples.

(c) *Preparation of Material for Microscopy*

(i) *Light Microscopy.*—Whole seeds or whole cotyledons were prepared for light microscopy as described previously (Bain and Mercer 1965).

(ii) *Electron Microscopy.*—Small pieces of tissue from the inner and outer part of the cotyledon were taken at each sampling and prepared for electron microscopy as described previously (Bain and Mercer 1966). The inner and outer tissue (separated in the region of the ring of vascular tissue) was treated separately throughout the investigation, but only cells of the inner tissue will be discussed here. Osmium tetroxide gave better preservation of subcellular organization and storage reserves in the cotyledons than potassium permanganate during germination and seedling development.

The form of the storage reserves (starch, protein, and fat) has been established already in electron micrographs of cells of developing pea cotyledons (Bain and Mercer 1966). Varner and Schidlovsky (1963) have shown the electron-dense material increasing with protein content in the developing pea cotyledon to be mainly globulin.

III. Results

Physiological data were collected for 3 weeks, but as brown lesions were appearing on the cotyledons after the 10th–12th day, it was considered that physiological activity in the tissue was influenced thereafter by the presence of pathogens.

Three morphological phases were identified in the development of the seedling, and these served as a basis for correlating the analytical and ultrastructural changes in the cotyledons (Fig. 1):

Phase 1: The first phase, lasting approximately 5 days, represented the period of development of the seedling from the initial imbibition of water by the seed until the radicle was approximately 40 mm long. Cell division commenced in the radicle after the fourth day and was very active by the fifth day. The shoot was still enclosed by the split testa at the end of phase 1.

Phase 2: This phase lasted from the fifth to the eighth day, during which time the shoot emerged from between the cotyledons and expanded to break the sand surface. The leaves unfolded and were green. The primary root continued to elongate during this phase and secondary root primordia appeared.

Phase 3: Phase 3 coincided with the further growth of the seedling from the 9th to the 22nd day. The cotyledons deteriorated considerably and the testa became slimy during this time.

(a) *Phase 1 in Seedling Development*

(i) *Morphology and Anatomy*

The seeds when planted were very wrinkled and the testas difficult to remove. The cotyledons had large reserves of starch and protein; fat content was considerable. These reserves were indicated by using iodine, mercuric bromphenol blue, and Sudan III.

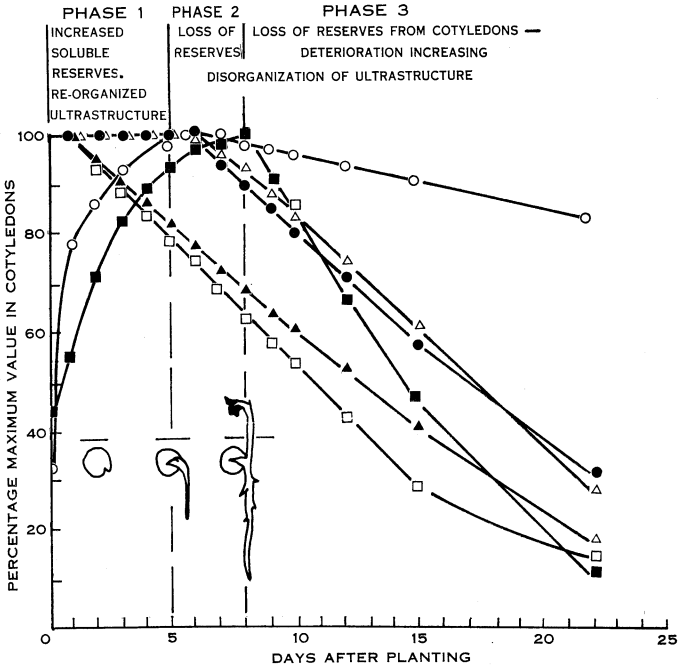


Fig. 1.—Changes in fresh weight (○), dry weight (●), total nitrogen (△), protein nitrogen (▲), starch (□), and sugar (■) in the cotyledons during germination of the pea seed and establishment of the seedling. Growth of the seedling has been divided into three morphological phases. Data are expressed as a percentage of their maximum value in the cotyledons during 22 days after planting.

One day after planting.—Testas were easily peeled from the cotyledons but some of the cotyledons were still wrinkled. Starch grains were very conspicuous and protein reserves were plentiful in light micrographs of the cotyledon cells. The limit of the nucleus was indistinct. The nucleolus had a distinct round outline.

Two days after planting.—The cotyledons were no longer wrinkled and the tip of the radicle had broken the testa close to the micropyle. Cell division was not yet occurring in the radicle.

Three days after planting.—The radicle was approximately 10 mm long and the testa had split further. Growth of the radicle was due to cell elongation, cell division still not having commenced in the root meristem. Nuclei had enlarged in the cotyledon cells and were somewhat lobed.

Four days after planting.—The radicle was about 20 mm long, but the shoot system was still enclosed by the testa. A few cell divisions were evident in the root tip.

Five days after planting.—The radicle was about 40 mm long at the end of phase 1 and cells were actively dividing in its tip. The shoot had begun to expand; the epicotyl was just protruding from between the cotyledons, but the leaves were still enclosed in the testa. Little difference was detected in the cytology of the cotyledon cells at the third and fifth day. Storage reserves (starch, protein, and fat) were plentiful in the cotyledon cells, with starch grains the most conspicuous feature under the light microscope.

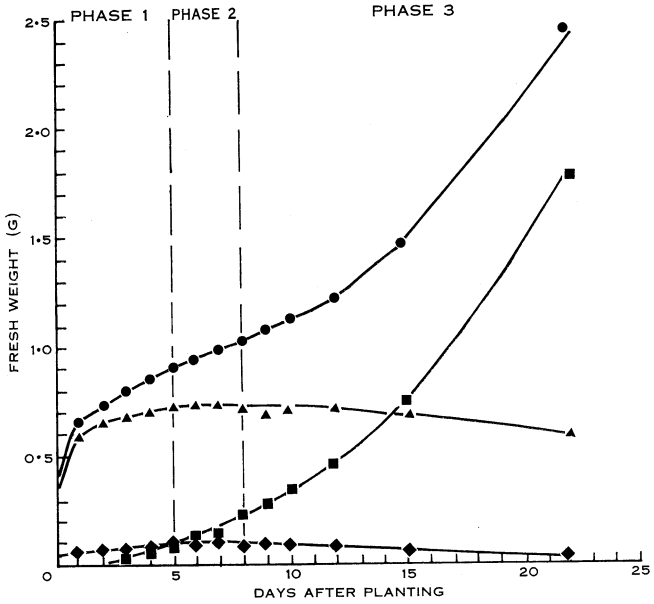


Fig. 2.—Changes in the average fresh weight of the seedling (●), axis (■), pair of cotyledons (▲), and the testa (◆) during the three morphological phases in development of the pea seedling.

(ii) *Physiological Data*

The results of the physiological changes occurring during phase 1 are given in Figures 1–6. Absorption of water by the cotyledons caused most of the increase in seedling weight during phase 1 (Fig. 2). There was little change in dry weight of the seedling or of its component parts (Fig. 3). Changes in storage reserves began with hydration of the tissue. Starch decreased as total sugar (approximately 90% reducing sugar) increased (Fig. 4). This increase in total sugar accounted for about 80% of the starch lost, and most of the remainder could be accounted for by the increase in respiration during phase 1 (Fig. 6). Protein nitrogen decreased but total nitrogen level did not alter (Fig. 5). Though part of the carbohydrate and nitrogen reserves were altered to more soluble forms during phase 1 in seedling development, they did not appear to be lost from the cotyledons to the axis.

(iii) *Ultrastructure*

The ultrastructure of dormant cells is not readily obtainable, owing to the methods necessarily used in fixation. Pea cotyledon tissue, being very compact, was difficult to penetrate with fixative, and imbibition began during this process. Electron micrographs of pea cotyledon tissue, taken as the seeds were drying out and wrinkling

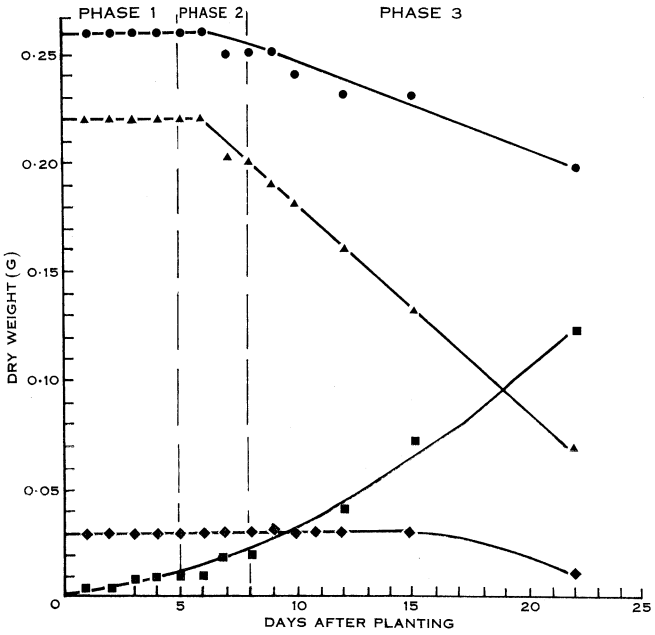


Fig. 3.—Changes in the average dry weight of the seedling (●), axis (■), pair of cotyledons (▲), and the testa (◆) during the three morphological phases in development of the pea seedling.

after 45 days on the vine, have been taken to give the approximate ultrastructure of the cells at planting (Plate 1, Figs. 1–3). Ultrastructural changes occurring in the cotyledons during phase 1 of seedling development were as follows:

One day after planting.—The general structure observed 24 hr after planting is shown in Plate 2, Figures 1 and 2. Numerous small vesicles were present in the cytoplasmic matrix (Plate 3, Fig. 1). Many mitochondria could be recognized only as membrane-bound organelles with no internal structure (Plate 3, Fig. 1); others showed some development of cristae (Plate 3, Fig. 2), though their internal structure was not developed fully. A few partly developed Golgi bodies were seen (Plate 3, Fig. 3). The nucleus appeared either rounded or somewhat lobed, and ribosome-like particles were aggregated in the nucleoplasm (Plate 2, Fig. 2). The "empty" space separating the starch grains from the cytoplasm of the cell approaching dormancy (Plate 1, Fig. 1) was no longer found in the tissue after planting, the starch grains then being embedded in the cytoplasm (Plate 3, Fig. 1). There was no development of a membrane to replace the plastid membrane that disappeared from around the starch grain as

dehydration occurred during ripening (Bain and Mercer 1966). In contrast, a distinct boundary separated the reserve protein from the cytoplasm (Plate 3, Fig. 2). Protein material was either diffused in this membrane-enclosed space (Plate 3, Fig. 2), or aggregated on the membrane (Plate 3, Fig. 1); the whole structure was regarded as the protein body. Fat was concentrated beneath the cell wall (Plate 3, Fig. 3) and in larger deposits throughout the cytoplasm (Plate 3, Fig. 1).

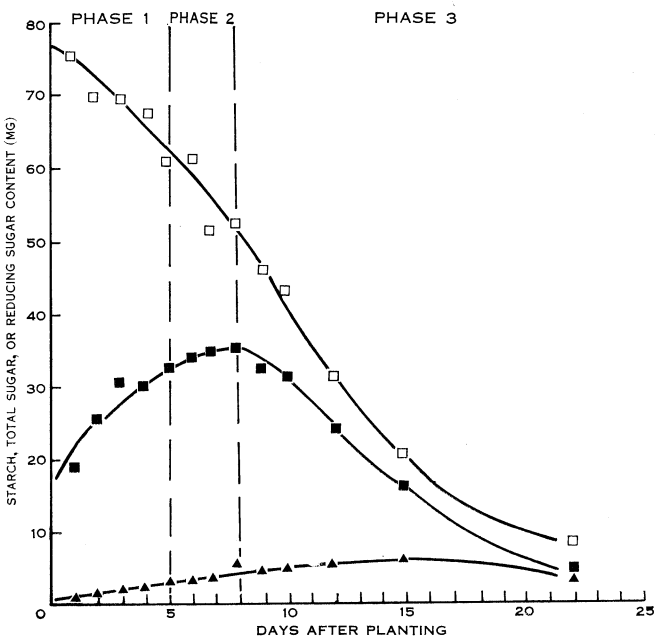


Fig. 4.—Changes in the starch (□), total sugar (■), and reducing sugar (▲) content per pair of cotyledons during the three morphological phases in development of the pea seedling.

Two days after planting.—The form of the storage bodies (Plate 4, Figs. 1 and 2) was still similar to that in day 1, but there seemed to be less protein in the protein bodies. Clustered vesicles, tending to be elongated, had formed in the cytoplasm (Plate 4, Figs. 1–3). These membranous structures were usually in close proximity to fat deposits (Plate 4, Figs. 1 and 3) and were especially concentrated beneath the cell wall (Plate 4, Fig. 3). Cristae were more developed in the mitochondria (Plate 4, Fig. 2) than at day 1; mitochondria were regarded now as having their complete structure. Organization of the Golgi bodies was now normal.

Three days after planting.—Vesicles were more frequent in the cytoplasm and many of these had fused to form a series of parallel pairs of membranes (Plate 5, Fig. 1).

Four days after planting.—The membrane system in the cytoplasm had increased by further fusion of vesicles and was conspicuous in the cell (Plate 5, Fig. 2). Little change was recognized in the protein bodies or starch grains during the first 4 days. Nuclei, now appearing larger in section, were distinctly lobed. Light micrographs confirmed the suggested enlargement of the nucleus.

Five days after planting.—The cytoplasm was permeated by a network of long, paired, parallel membranes (endoplasmic reticulum) at the end of phase 1 (Plate 5, Fig. 3). The cytoplasm was granular, but ribosomes were not so easily distinguished as when the cells were synthesizing storage protein during development of the cotyledons (Bain and Mercer 1966). Fewer large starch grains were seen in the sections. Protein bodies and accumulations of fat were prominent in the cytoplasm (Plate 5, Fig. 3). Nuclei had enlarged greatly, were very lobed (confirmed by light microscope observations), and particles were aggregated in the nucleoplasm (Plate 6, Fig. 1).

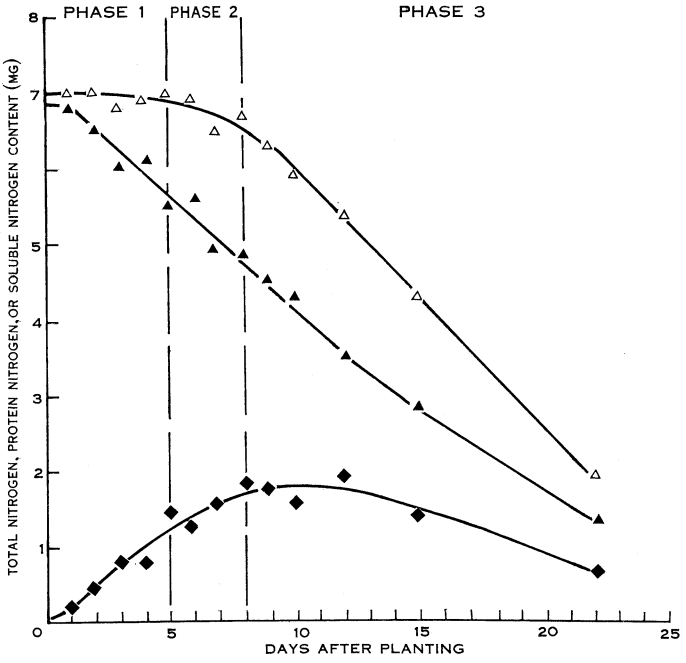


Fig. 5.—Changes in total nitrogen (△), protein nitrogen (▲), and soluble nitrogen (◆) content per pair of cotyledons during three morphological phases in development of the pea seedling.

(*b*) *Phase 2 in Seedling Development*

(i) *Morphology and Anatomy*

The shoot system became free of the cotyledons and the testa after the fifth day and began to expand.

Six days after planting.—The shoot was approximately 10 mm long; the tip was hooked and the folded leaves yellow. The root was approximately 50 mm long. A central depression had formed in the inner flat surface of the cotyledons, but they were still firm.

Seven days after planting.—The hooked shoot system continued to expand towards the sand surface and was tinged with green. The root was now more than 60 mm long and a few secondary root primordia had developed.

Eight days after planting.—The shoot broke through the sand surface, the apex straightened, and leaves became green. The main root was over 80 mm long and secondary roots were developing. Cotyledons, though often sunken and sometimes cracked in the central region, remained firm and were still covered by the testa. Starch, protein, and fat deposits were still plentiful at the end of phase 2. Nuclei were very lobed.

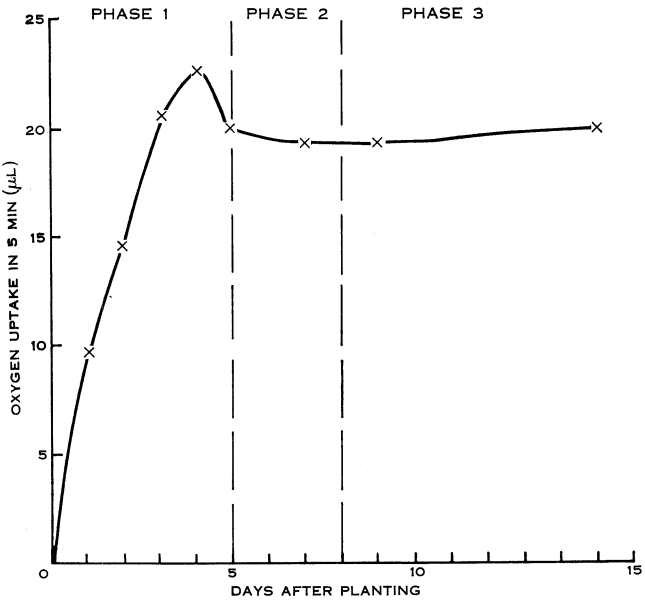


Fig. 6.—Changes in respiration rate in the cotyledons during the three morphological phases in development of the pea seedling.

(ii) *Physiological Data*

The results of the physiological changes occurring in the cotyledons during phase 2 are shown in Figures 1–6. Transport of soluble carbohydrate and nitrogen from the cotyledons to the axis began during this phase. Starch continued to decrease as in phase 1, but total sugar increased at a slower rate than in phase 1 (Fig. 4). Protein nitrogen decreased at the same rate as in phase 1, loss of total nitrogen indicating movement of the soluble fraction from the cotyledons (Fig. 5).

(iii) *Ultrastructure*

The membrane systems which became organized in phase 1 (endoplasmic reticulum, Golgi bodies, mitochondria) became disorganized again during phase 2 (Plate 6, Fig. 2; Plate 7, Figs. 1 and 2). The form of the endoplasmic reticulum was less regular by the sixth day (Plate 7, Fig. 1) and by the eighth day the long, paired, parallel membranes had broken into smaller segments or vesicles (Plate 7, Fig. 2). The cristae of mitochondria were disorganized by the eighth day, but the outer double-membrane structure was clearly resolved (Plate 7, Fig. 2). Ribosome-like structures and disorganized Golgi bodies were evident in the cytoplasm at the eighth

day (Plate 7, Fig. 2). Nuclear structure was less lobed at the end of phase 2. Fat deposits appeared to have increased in size and number with the breakdown of the membrane systems (Plate 7, Fig. 2). Starch and protein reserves were much scarcer in the sectioned material during phase 2. No limiting membrane was observed around the starch grains (Plate 7, Fig. 1) and starch grains appeared smaller. Protein material often appeared clumped in the protein body or frequently appeared "vacuolated" as if the aggregate was being broken down internally (Plate 6, Fig. 2; Plate 8, Fig. 1). These bodies were bounded by a distinct membrane in early phase 2. Protein material decreased in the protein body during phase 2, and at the end of this phase their membranes were not resolved so distinctly (Plate 7, Fig. 2; Plate 8, Fig. 1).

(c) *Phase 3 in Seedling Development*

(i) *Morphology and Anatomy*

Depressions had developed in the inner surface of most of the cotyledons by the beginning of phase 3, and brown lesions, associated with the development of pathogens, were appearing on the outer surface of many of them after the 10th day. Thereafter, the cotyledons deteriorated rapidly. Anatomical observations, limited to the early part of phase 3, showed that the starch grains had become smaller and fewer in number. The nuclei appeared rounder in outline. Histochemical tests indicated that starch, protein, and fat were present 3 weeks after planting.

(ii) *Physiological Data*

Physiological data covering this period are shown in Figures 1–6. These data were collected for 3 weeks, but those for the period after the 10th–12th day are for cotyledons that showed considerable deterioration.

Analytical data indicated that in the 3 weeks following planting of the seed the axis did not utilize all the reserve material in the cotyledons as the seedling became an established plant. The level of starch in the deteriorating cotyledon tissue was determined, but the amount of reserve protein left was not known. Other data for germinating pea seeds indicated that this would be a very small fraction of the total protein present in the tissue 3 weeks from planting (Danielsson 1951).

(iii) *Ultrastructure*

The disorganization of ultrastructure continued into phase 3. Many of the small vesicles resulting from the breakdown of the endoplasmic reticulum system fused to form large vesicles (Plate 8, Fig. 2). Mitochondria were further disorganized than in phase 2; their internal structure was obscure, but there were still some indications of double structure of the external membranes (Plate 8, Fig. 2). Fat deposits were plentiful as phase 3 progressed (Plate 8, Fig. 2), but starch grains and protein material were difficult to locate. Nuclear structure was not identified during this phase. The cytoplasm was highly vesiculated 3 weeks from planting; mitochondria were recognized from their size, shape, and possible membrane structure, and deposits of fat were frequent in the cell (Plate 8, Fig. 3).

IV. Discussion

The development of the complex subcellular organization in the cotyledons during phase 1 of seedling development seems to be correlated rather with the

absorption of water by the cotyledon tissue than with the catabolic processes characteristic of the tissue, and maximum ultrastructural development coincided with maximum water content of the cells at the end of this phase. Breakdown of starch and protein reserves continued at constant rates throughout phase 1, even though the cotyledons were still absorbing water and the ultrastructure was still being organized in the cells. Disorganization of endoplasmic reticulum, formation of vesicles in the cytoplasm, and loss of mitochondrial structure are characteristic of aging tissue (Bain and Mercer 1964) and disorganization of ultrastructure observed early in phase 3 thus signalled approaching senescence. The increased vacuolation of the cytoplasm and the swelling of mitochondria possibly arose from increased permeability of cell membranes.

The formation of the endoplasmic reticulum in the first few days after planting coincided with gradually increasing water content in the pea cotyledon and with the disappearance of fat from the cytoplasm. It is possible, therefore, that the fat material was used directly in the formation of the membrane system and that the vesicles separated as a new phase as the water content of the cytoplasm increased. The fat, accumulations of which coincided with the disappearance of membranes as the seeds passed into dormancy (Bain and Mercer 1966), may have constituted a reserve to be used in the formation of a new system of membranes after the breaking of dormancy. The endoplasmic reticulum appeared to be a separate membrane system in the cell and no connexions were observed between it and the nuclear membrane or the plasmalemma.

The role of the endoplasmic reticulum is not understood. It was tempting to postulate that it may have assisted the intracellular transport of substances, since the vascular tissue was not extensive in the cotyledon and many cells were up to 20 cells away from the vascular supply or from the axis; but the soluble reserves were not lost from the cotyledons during this time, so this role seemed unlikely. The correlation observed between the development of the endoplasmic reticulum and the accumulation of soluble carbohydrate, if not fortuitous, could mean that the membrane system was functioning as a vacuolar system for the temporary storage of soluble reserves (especially sucrose) during phase 1, since their transport from the cotyledons to the axis did not commence until phase 2. This coincided with considerable loss of ultrastructural organization. During phases 2 and 3 the seedling axis must have been a very efficient physiological sink that eliminated problems of transport, since while developing it was in close contact with a tissue whose cell contents were becoming disorganized. Also, it is possible that individual cells which became disorganized during the development of the cotyledons (Bain and Mercer 1966), and which were recognizable again in the cotyledons during phase 1 of seedling development, may have served as channels for the mass movement of substances through the cotyledons.

Respiration rate increased with increasing water content of the seed, confirming the findings of Opik and Simon (1963). Respiration rate was also linked with the reorganization of mitochondrial structure. The origin of the mitochondria developing in cotyledon cells during the 2 days after planting is not known. They could have arisen from pre-existent mitochondria that were not resolvable in the cotyledons that were approaching dormancy; or from "ghosts" of mitochondria which had lost their enzymic machinery as cells became dormant (pro-mitochondria); or, finally, they may

have arisen *de novo* as the cytoplasm became hydrated. As biochemical data on mitochondria isolated from seeds indicate that synthesis of mitochondrial components can occur during germination (Akazawa and Beevers 1957*a*, 1957*b*; Young *et al.* 1960; Cherry 1963) an origin from pro-mitochondria, or *de novo*, seems the most likely. The gradual loss of mitochondrial structure was not associated with a fall in respiration rate per pair of cotyledons during phase 2 (Fig. 6), though there was a slight fall in rate on a fresh weight basis. Respiration rate rose as phase 3 progressed, even though mitochondrial structure appeared rather disorganized, and this was possibly due to increased availability of substrates in the cotyledon cells with increased permeability of membranes as senescence progressed; it may also have been partly due to the presence of pathogens on the cotyledons. The observed loss of mitochondrial structure parallels other existing biochemical data for pea and other germinating seeds. Young *et al.* (1960) isolated active mitochondria from pea cotyledons up to the 10th day of planting, and Huang (1960), cited by Varner (1961), isolated a soluble, heat-labile, dialysable substance (not ribonuclease) that produced swelling and uncoupling of mitochondria isolated from pea cotyledons 10–13 days after planting. Loss of mitochondrial activity has been associated with ribonuclease activity, this enzyme being responsible for making holes in mitochondrial membranes (Hanson 1959, 1961). Cherry (1963) showed that mitochondrial activity decreased and their structure became disorganized in peanut cotyledons 8 days after planting; this disorganization coincided with a sevenfold increase in ribonuclease activity.

It is unlikely that the slow recovery of respiration was due to an initial shortage of substrates limiting respiration rate after planting, as the concentration of sugar is high even in dry cotyledons (approximately 20 mg per seed). High respiratory quotients have been found in pea seeds soon after planting (Spragg and Yemm 1959; Varner, Balce, and Huang 1963). Such values would be dependent on the impermeability of the testa to oxygen in the early period of germination and could also be related to the possibility that enzymes of the glycolytic cycle (possibly cytoplasmic) become active before the oxidative enzymes (possibly situated on mitochondrial cristae—Férnandez-Morán 1962*a*, 1962*b*; Férnandez-Morán *et al.* 1964). Gradual increases in oxygen uptake with increasing development of mitochondrial structure in the present observation was consistent with this pattern.

Biochemical observations on the pea cotyledon (Young 1957*a*, 1957*b*; Young and Varner 1959; Young *et al.* 1960; Barker and Douglas 1960; Varner, Balce, and Huang 1963) have shown synthesis to occur following the breaking of dormancy, and such processes imply the presence of appropriate ribosomes in the cells. It is not known whether these ribosomes would arise from pre-existing ribosomes that cannot be resolved during dormancy, of whether they would arise *de novo* as nuclear activity recovers during germination.

Ribosome-like particles could be distinguished in the nuclei 1 day after planting in the present study, but nuclear activity (assessed from the increased size and marked lobing) was greatest at the end of phase 1. It is possible, therefore, that there may be two groups of ribosomes in the cells in phase 1, some forming during the development of the seeds and pre-existing in the cytoplasm, and some forming during the recovery of nuclear activity on germination. If this were so, the first group could be involved

in the synthesis of enzymes from amino acids derived from the breakdown of the protein reserves during the first few days of germination, and the second group could be involved in subsequent synthetic processes, particularly at the end of phase 1. It would clearly not be possible to decide which group of ribosomes are involved in the rapid increase of phosphatase that occurs in the first 5 days of germination (Young 1957*a*, 1957*b*) or in the increase amylase observed after the first 3 days of germination (Young and Varner 1959). Nor would it be possible to decide which group of ribosomes would correspond with the microsomal fraction isolated from cotyledons of germinating pea seeds and shown to be capable of incorporating [^{14}C]glycine into protein *in vivo* and *in vitro* (Young and Varner 1959). Ribosomes could not always be identified with certainty in the electron micrographs of the present cotyledon tissue, but their number appeared to be far fewer than in cells of developing cotyledons (Bain and Mercer 1966).

Changes in the amount of storage reserves and the manner in which they were degraded were not readily apparent from electron micrographs during phase 1. In some instances the starch grain had a wavy outline, suggesting intensive localized enzyme attack (Plate 3, Fig. 1); in others the outline appeared smoother, suggesting that enzyme attack could be taking place more evenly over the surface (Plate 4, Fig. 1). The absence of a membrane around the starch grains in the hydrated cotyledon cells must be an important factor in the functioning of the degradative enzymes in the cytoplasm of these cells. On the other hand, the partitioning off of the protein material from the cytoplasm by the membrane of the protein body seems to be an important feature of cell structure: the localization of proteolytic enzymes within such a membrane would prevent them from attacking the cytoplasmic proteins during germination. The membrane of the protein body was resolved very clearly on the first day after planting the seed.

The presence of limiting membranes for the protein body means that the soluble products of breakdown are not released directly into the cytoplasm. As breakdown proceeds, the soluble products must accumulate within the protein bodies and then "leak" through the membranes into the cytoplasm. It seems likely that the proteolytic enzymes are synthesized at the same time as the reserve material during development of the seed. If formed in early germination, the enzymes would have to be transported from the sites of synthesis through the membranes and into the interior of the protein body very soon after planting. Young and Varner (1959) showed that the protease activity remains constant in pea cotyledons during the first 8 days after planting; this implies that proteolytic enzymes are not synthesized during germination, but during the development of the cotyledons. If proteolytic enzymes are indeed formed at the same time as the reserve protein, they might conceivably be secreted in an inactive form, becoming active as the seeds dry and pass into dormancy, or when the dry cotyledons imbibe water.

V. Acknowledgments

We wish to thank Misses Rosemary Mullens and Barbara Williams for assistance with section cutting, and Mr. J. Smydzuk for the analytical data on prepared material.

VI. References

Akazawa, T., and Beevers, H. (1957*a*).—Some chemical components of mitochondria and microsomes from germinating castor beans. *Biochem. J.* **67**:110–14.

Akazawa, T., and Beevers, H. (1957*b*).—Mitochondria in the endosperm of germinating Castor bean; a developmental study. *Biochem. J.* **67**: 115–18.

Bain, Joan M. (1964).—The relation of subcellular organization to some metabolic processes in plant cells. Ph.D. Thesis, University of Sydney.

Bain, Joan M., and Mercer, F. V. (1964).—Organization resistance and the climacteric. *Aust. J. Biol. Sci.* **17**:78–85.

Bain, Joan M., and Mercer, F. V. (1966).—Subcellular organization of the developing cotyledons of *Pisum sativum* L. *Aust. J. Biol. Sci.* **19**: 49–67.

Barker, G. R., and Douglas, T. (1960).—Function of ribonuclease in germinating peas. *Nature, Lond.* **188**: 943–4.

Cherry, J. H. (1963).—Nucleic acid, mitochondria and enzyme changes in cotyledons of peanut seeds during germination. *Plant Physiol.* **38**: 440–6.

Crocker, W., and Barton, L. V. (1953).—"Physiology of Seeds." (Chronica Botanica: Waltham, Mass.)

Danielsson, C. E. (1951).—The breakdown of high molecular reserve proteins of peas during germination. *Acta Chem. Scand.* **5**: 541–54.

Férnandez-Morán, H. (1962*a*).—"New Approaches to the Study of Biological Ultrastructure by High Resolution Electron Microscopy." (Ed. R. J. C. Harris.) (Symp. Int. Soc. Cell Biol.) Vol. 1. pp. 411–27. (Academic Press, Inc.: New York.)

Férnandez-Morán, H. (1962*b*).—Cell membrane ultrastructure. Low temperature electron microscopy and X-ray diffraction studies of lipoprotein components in lamellar systems. *Circulation* **26**: 1039–65.

Férnandez-Morán, H., Oda, T., Blair, P. V., and Green, D. E. (1964).—A macromolecular repeating unit of mitochondrial structure and function. Correlated electron microscopic and biochemical studies of isolated mitochondria and sub-mitochondrial particles of beef heart muscle. *J. Cell Biol.* **22**: 63–100.

Hanson, J. B. (1959).—The effect of ribonuclease on oxidative phosphorylation by mitochondria. *J. Biol. Chem.* **234**: 1303–6.

Hanson, J. B. (1961).—Studies on the uncoupling action of ribonuclease applied to corn shoot mitochondria *Plant Physiol.* **36**: xxxii.

Hoagland, D. R., and Arnon, D. I. (1939).—Water culture method for growing plants without soil. Circ. Calif. Agric. Exp. Stn. No. 347.

Huang, R. C. (1960).—The cause of cellular senescence and its prevention. Ph.D. Thesis, Ohio State University, Columbus, Ohio. [Cited by Varner (1961).]

Koller, D., Mayer, A. M., Poljakoff-Mayber, A., and Klein, S. (1962).—Seed germination. *Ann. Rev. Pl. Physiol.* **13**: 437–64.

Malhotra, R. C. (1933).—Biochemical study of seeds during germination. I. Periodic changes of reserve materials in normal germinating seeds. *Beih. Bot. Zbl.* **50**: 1–7.

Mayer, A. M., and Poljakoff-Mayber, A. (1963).—"The Germination of Seeds." (Int. Series of Monographs on Pure and Applied Biology. Division: Plant Physiology 3.) (Permagon Press: London.)

Opik, H., and Simon, E. W. (1963).—Water content and respiration rate of bean cotyledons. *J. Exp. Bot.* **14**: 299–310.

Spragg, S. P., and Yemm, E. W. (1959).—Respiratory mechanisms and the changes of glutathione and ascorbic acid in germinating peas. *J. Exp. Bot.* **10**: 409–25.

Toole, E. H., Hendricks, S. B., Borthwick, H. A., and Toole, V. K. (1956).—Physiology and seed germination. *Ann. Rev. Pl. Physiol.* **7**: 299–324.

Varner, J. E. (1961).—Biochemistry of senescence. *Ann. Rev. Pl. Physiol.* **12**: 245–64.

Varner, J. E., Balce, L. V., and Huang, R. C. (1963).—Senescence of cotyledons of germinating peas. Influence of axis tissue. *Plant Physiol.* **38**: 89–92.

Varner, J. E., and Schidlovsky, G. (1963).—Intracellular distribution of proteins in pea cotyledons. *Plant Physiol.* **38**: 139–44.

YOUNG, J. L. (1957*a*).—Enzyme synthesis in the cotyledons of germinating seeds. *Fed. Proc.* **16**: 275.

YOUNG, J. L. (1957*b*).—Conditions affecting enzyme synthesis in the cotyledons of germinating peas. *Plant Physiol.* **32** (suppl.): xxv.

YOUNG, J. L., and VARNER, J. E. (1959).—Enzyme synthesis in the cotyledons of germinating seeds. *Arch. Biochem. Biophys.* **84**: 71–8.

YOUNG, J. L., HUANG, R. C., VANECKO, S., MARKS, J. D., and VARNER, J. E. (1960).—Conditions affecting enzyme synthesis in cotyledons of germinating seeds. *Plant Physiol.* **35**: 288–92.

EXPLANATION OF PLATES 1–8

All plates are electron micrographs of pea cotyledon tissue fixed in buffered 1% osmium tetroxide, stained with uranyl acetate, embedded in Araldite, and sectioned

PLATE 1

Figures 1, 2, and 3 represent the ultrastructure of cotyledons as the seeds were ripening on the vine after 45 days' development. The organization in these cells is assumed to represent the approximate structure of dormant cells at the time of planting.

Fig. 1.—The cytoplasm (*Cyt*) is undifferentiated and vesiculated. Protein bodies (*PB*) are round and in many cases the surrounding membrane is quite distinct. A starch grain (*SG*) is shown separated from the cytoplasm, but there is no evidence of a surrounding plastid membrane. Fat material (*F*) is obvious in the cytoplasm. ×10,000.

Fig. 2.—More detailed representation of the structure of the dormant cell. Membrane-bound protein bodies (*PB*) are obvious in the cytoplasm (*Cyt*). Mitochondria (*M*), though becoming disorganized, can be distinguished. Fat deposits (*F*) are especially concentrated beneath the cell wall (*CW*). ×20,000.

Fig. 3.—The nucleus (*N*) appears rather lobed in this instance. The structure of the cytoplasm and storage reserves is similar to that in Figure 1. ×9000.

PLATE 2

Fig. 1.—One day after planting. The cytoplasmic matrix (*Cyt*) appears undifferentiated. Reserve protein is diffuse in the membrane-bound protein bodies (*PB*). Mitochondria (*M*) are recognizable in the cytoplasm. Fat deposits (*F*) are especially concentrated beneath the cell wall (*CW*). ×10,000.

Fig. 2.—One day after planting. Shows the lobing of the nucleus (*N*) and the ribosome-like particles within it. The form of the protein bodies (*PB*) and the starch grain (*SG*) are shown. No membrane marks the limit of the starch grain. ×7500.

PLATE 3

Fig. 1.—One day after planting. The cytoplasmic matrix (*Cyt*) is undifferentiated except for a few small vesicles (*V*). Mitochondria (*M*) are distinguishable by their size, shape, and external membrane. Protein material is aggregated on the membranes of the protein bodies (*PB*). Starch grains (*SG*) are in close contact with the cytoplasm and show an irregular outline. Fat deposits (*F*) are concentrated in the cytoplasm. ×10,000.

Fig. 2.—One day after planting. Shows more detail of the cytoplasmic matrix (*Cyt*). Cristae are developing in the mitochondria (*M*). Protein bodies (*PB*) have a very distinct outer membrane; protein material appears diffuse. ×20,000.

Fig. 3.—One day after planting. A Golgi body (*GB*) is developing in the undifferentiated cytoplasm (*Cyt*). Fat deposits (*F*) are concentrated beneath the cell wall (*CW*). ×25,000.

PLATE 4

Fig. 1.—Two days after planting. Small vesicles (*V*) are forming in close proximity to the fat deposits (*F*) in the cytoplasm (*Cyt*). No distinct membrane separates the starch grain (*SG*), which has a smooth outline, from the cytoplasm. Protein material appears to have decreased in the protein bodies (*PB*). ×20,000.

Fig. 2.—Two days after planting. Mitochondria (*M*) are now fully organized. Protein material appears diffuse in the protein body (*PB*), which has a distinct surrounding membrane. Small vesicles (*V*) are developing in the cytoplasm (*Cyt*). ×20,000.

Fig. 3.—Two days after planting. Vesicles (*V*) are forming in the cytoplasm (*Cyt*), especially beneath the cell wall (*CW*), in close association with fat deposits (*F*). Protein bodies (*PB*) are also shown. ×15,000.

PLATE 5

Fig. 1.—Three days after planting. Vesicles (*V*) have continued to form in the cytoplasm (*Cyt*), and many of these have fused to form a membranous system. Fat deposits (*F*), beneath the cell wall (*CW*), are closely associated with this system. ×18,000

Fig. 2.—Four days after planting. Further development of the membranous system formed by fusion of vesicles in the cytoplasm (*Cyt*) is evident. Protein bodies (*PB*) are shown. ×12,500.

Fig. 3.—Five days after planting. The vesicles in the cytoplasm (*Cyt*) have fused to form a conspicuous endoplasmic reticulum (*ER*). The reticulum is made up of long, paired, parallel, smooth membranes. Mitochondria (*M*) and fat deposits (*F*) are shown in the cytoplasm. Protein material is diffuse in the protein body (*PB*). It is difficult to establish the presence of ribosomes in this granular matrix. ×20,000.

PLATE 6

Fig. 1.—Five days after planting. The nucleus (*N*) is large and very lobed. Ribosome-like particles are aggregated in the nucleoplasm. Mitochondria (*M*), endoplasmic reticulum (*ER*), and fat deposits (*F*) are seen in the cytoplasm (*Cyt*). ×15,000.

Fig. 2.—Six days after planting. The organization built up in the cell during phase 1 of seedling development is now beginning to break down. The endoplasmic reticulum (*ER*) is breaking down into smaller segments in the cytoplasm (*Cyt*). Mitochondria (*M*) are losing their organization. Much of the protein material in the protein bodies (*PB*) appears "vacuolated". ×10,000.

PLATE 7

Fig. 1.—Six days after planting. Fat deposits (*F*) are prevalent in the cytoplasm (*Cyt*) and the endoplasmic reticulum (*ER*) is breaking down into smaller segments or vesicles. No limiting membrane is observed around the starch grain (*SG*). Mitochondria (*M*) are becoming disorganized. ×18,000.

Fig. 2.—Eight days after planting. The endoplasmic reticulum has broken down into small vesicles (*V*) in the cytoplasm (*Cyt*). Cristae structure in the mitochondria (*M*) is disorganized, but the external double-membrane structure is still evident. Ribosome-like particles (*R*) are observed in the cytoplasm, also increasing amounts of fat (*F*). The surrounding membrane of the protein body (*PB*) is not very distinct at this stage. A Golgi body (*GB*) is becoming disorganized. ×22,500.

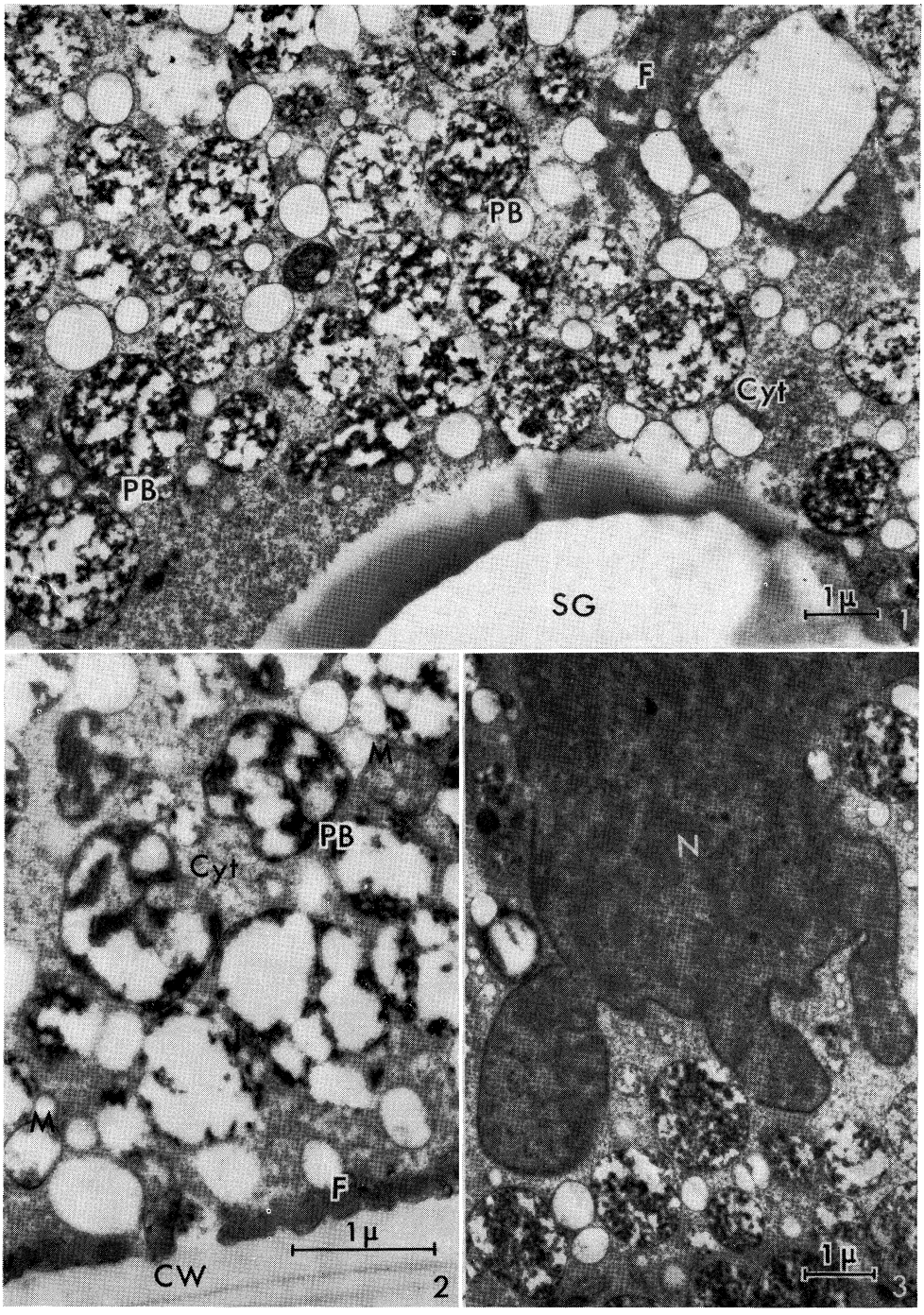
PLATE 8

Fig. 1.—Eight days after planting. Protein material in the protein body (*PB*) appears "vacuolated" and the membrane surrounding the body is less clearly resolved. The endoplasmic reticulum has broken down into a series of small vesicles (*V*). The internal structure of the mitochondria (*M*) is disorganized; the outer double-membrane structure is preserved. Fat deposits (*F*) are obvious in the cytoplasm (*Cyt*). ×15,000.

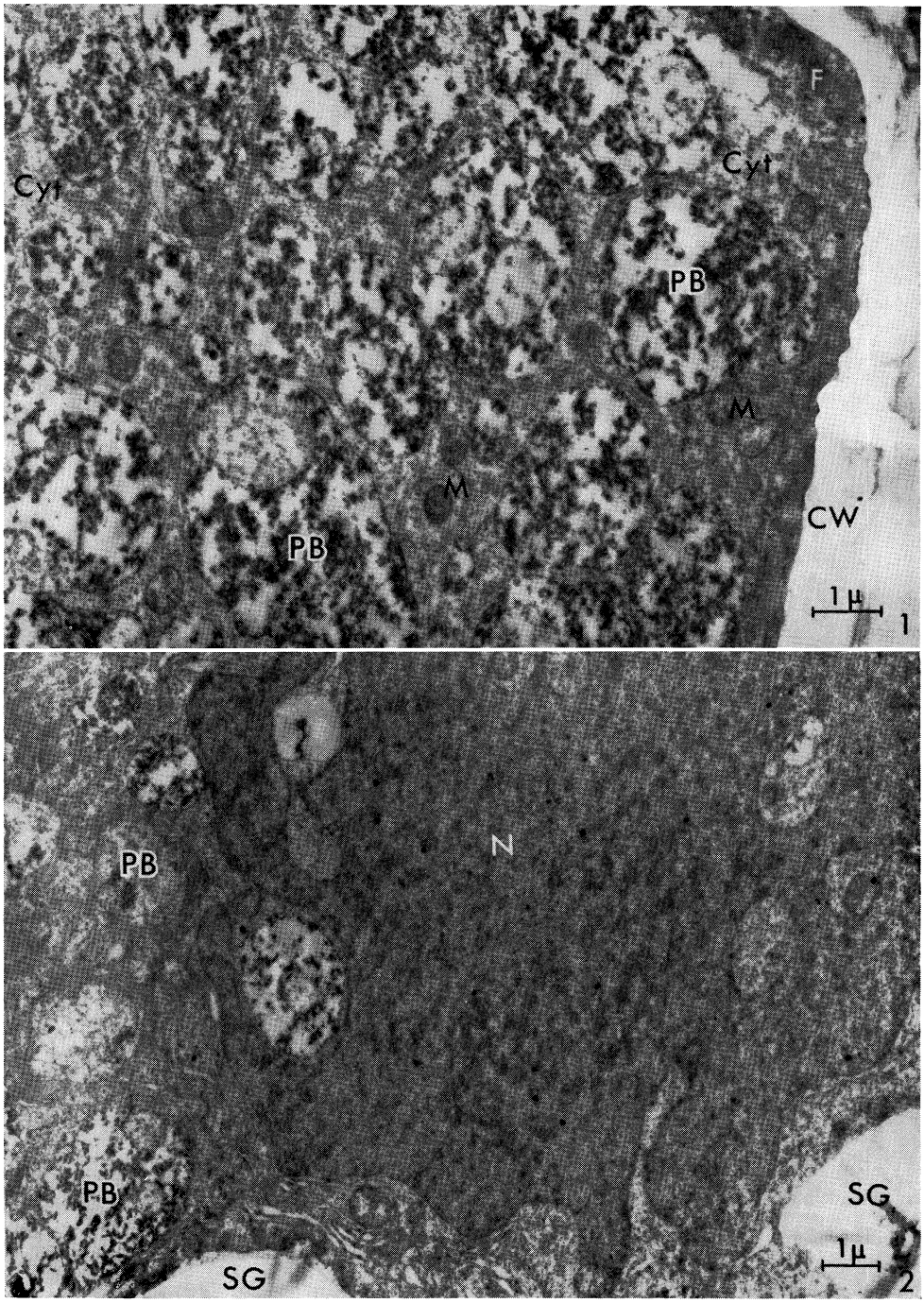
Fig. 2.—14 days after planting. Structure is now very disorganized. The cytoplasm (*Cyt*) is a mass of small and enlarged vesicles (*V*). Mitochondria (*M*), though disorganized, are still recognizable. Fat deposits (*F*) are very conspicuous. ×22,500.

Fig. 3.—22 days after planting. Shows ultrastructure characteristic of senescence. Disorganized mitochondria (*M*) are still recognizable in the vesiculated cytoplasm (*Cyt*). Fat deposits (*F*) are prominent. ×20,000.

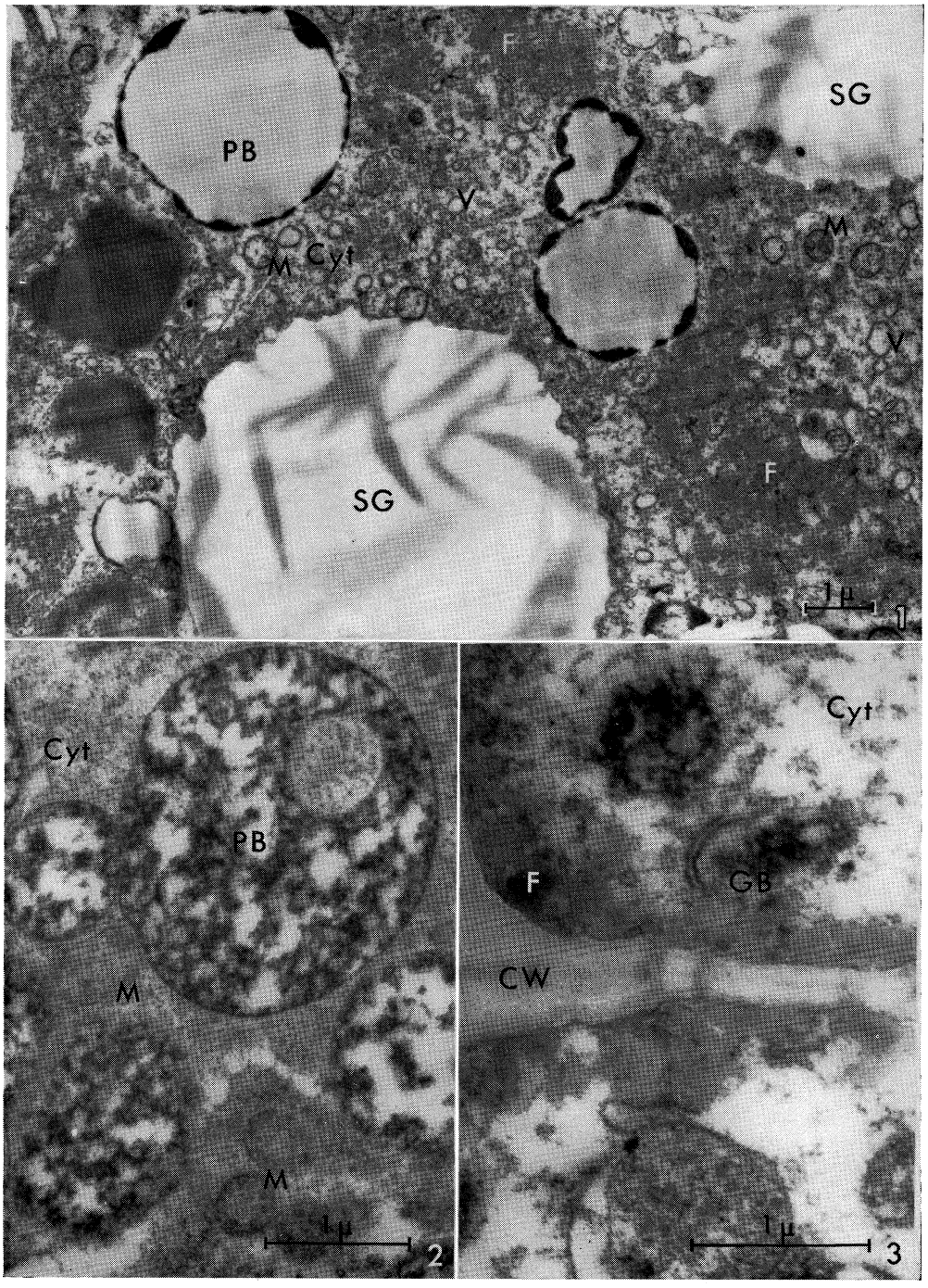
PEA COTYLEDONS DURING AND AFTER GERMINATION



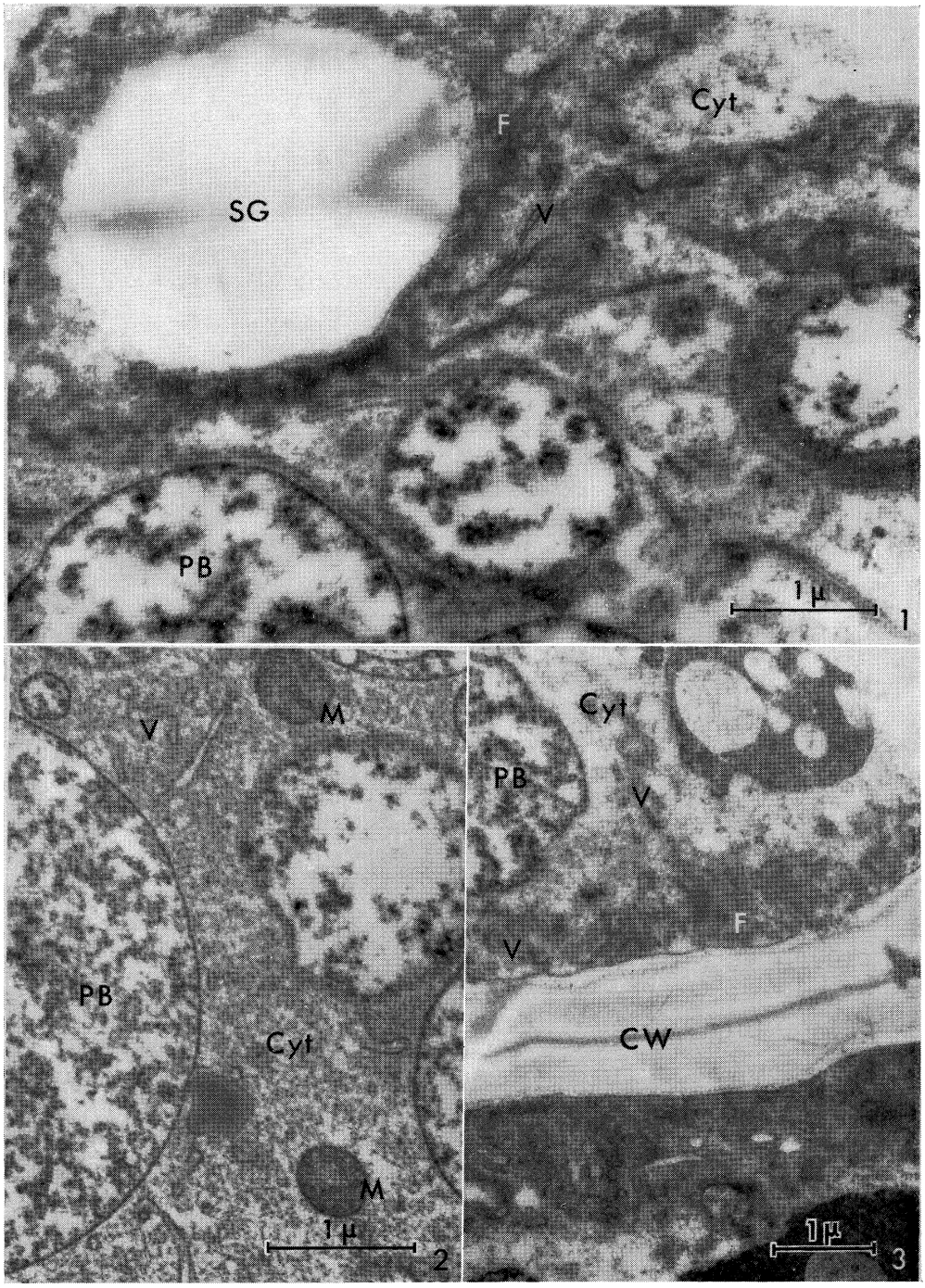
PEA COTYLEDONS DURING AND AFTER GERMINATION



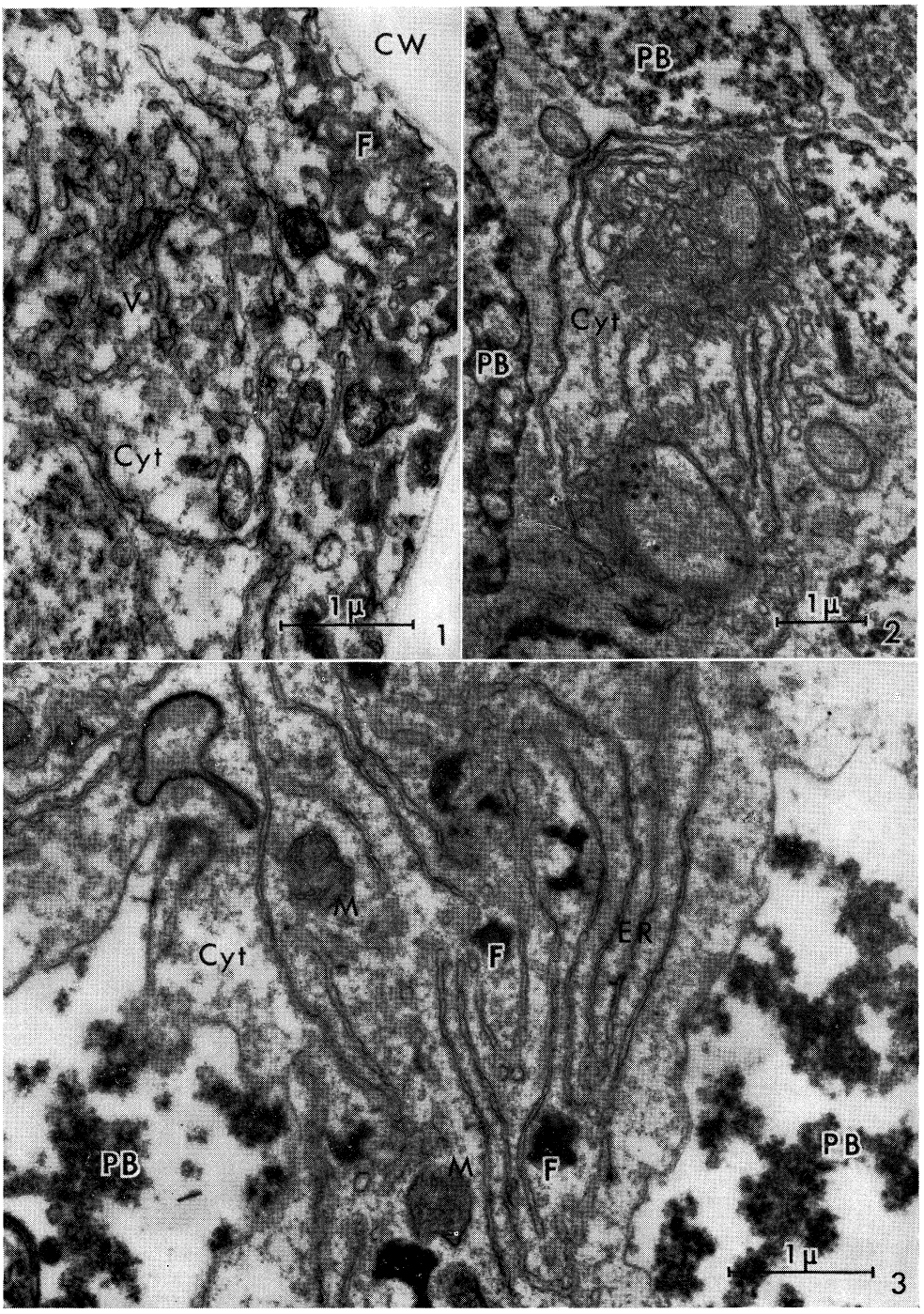
PEA COTYLEDONS DURING AND AFTER GERMINATION



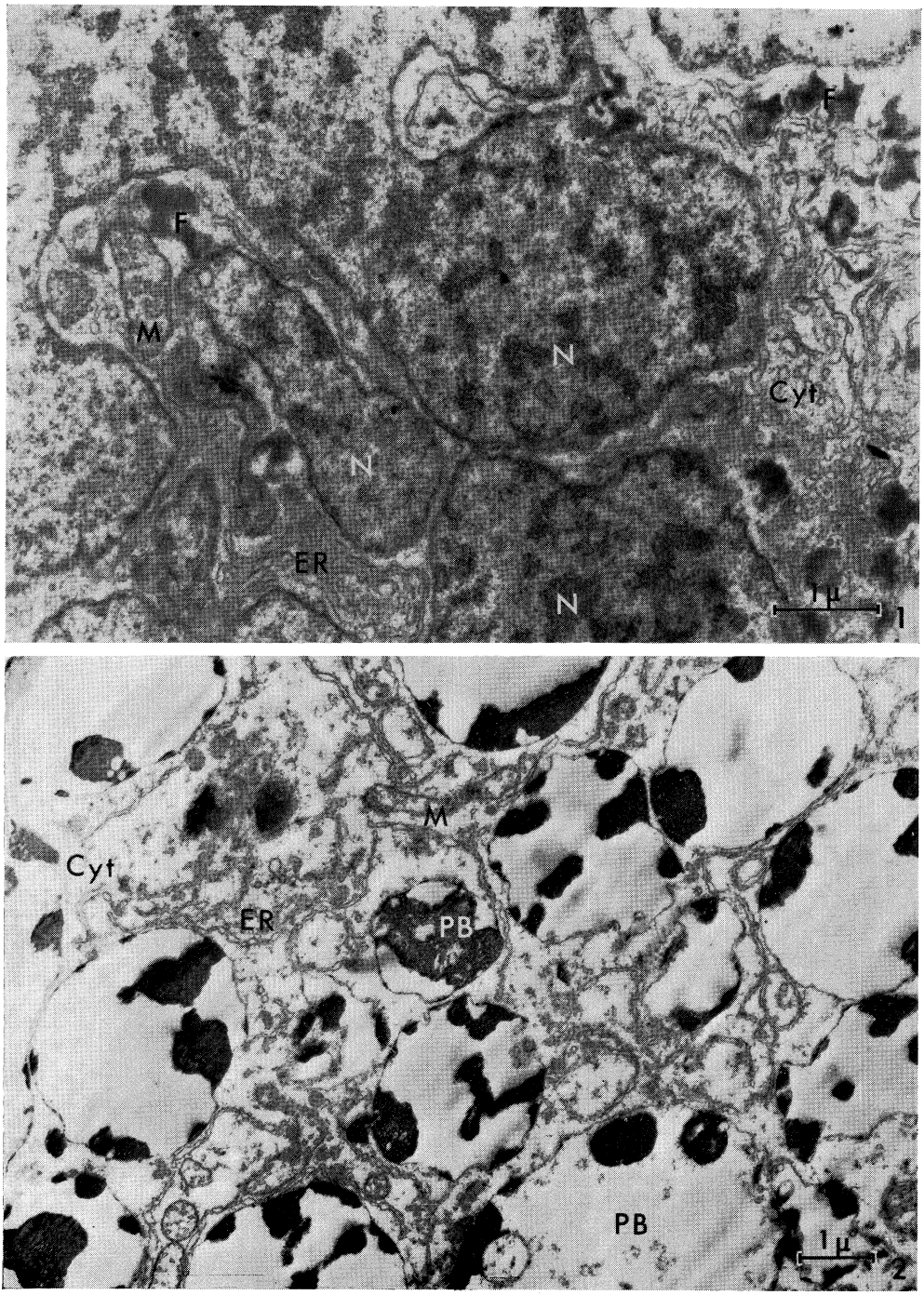
PEA COTYLEDONS DURING AND AFTER GERMINATION



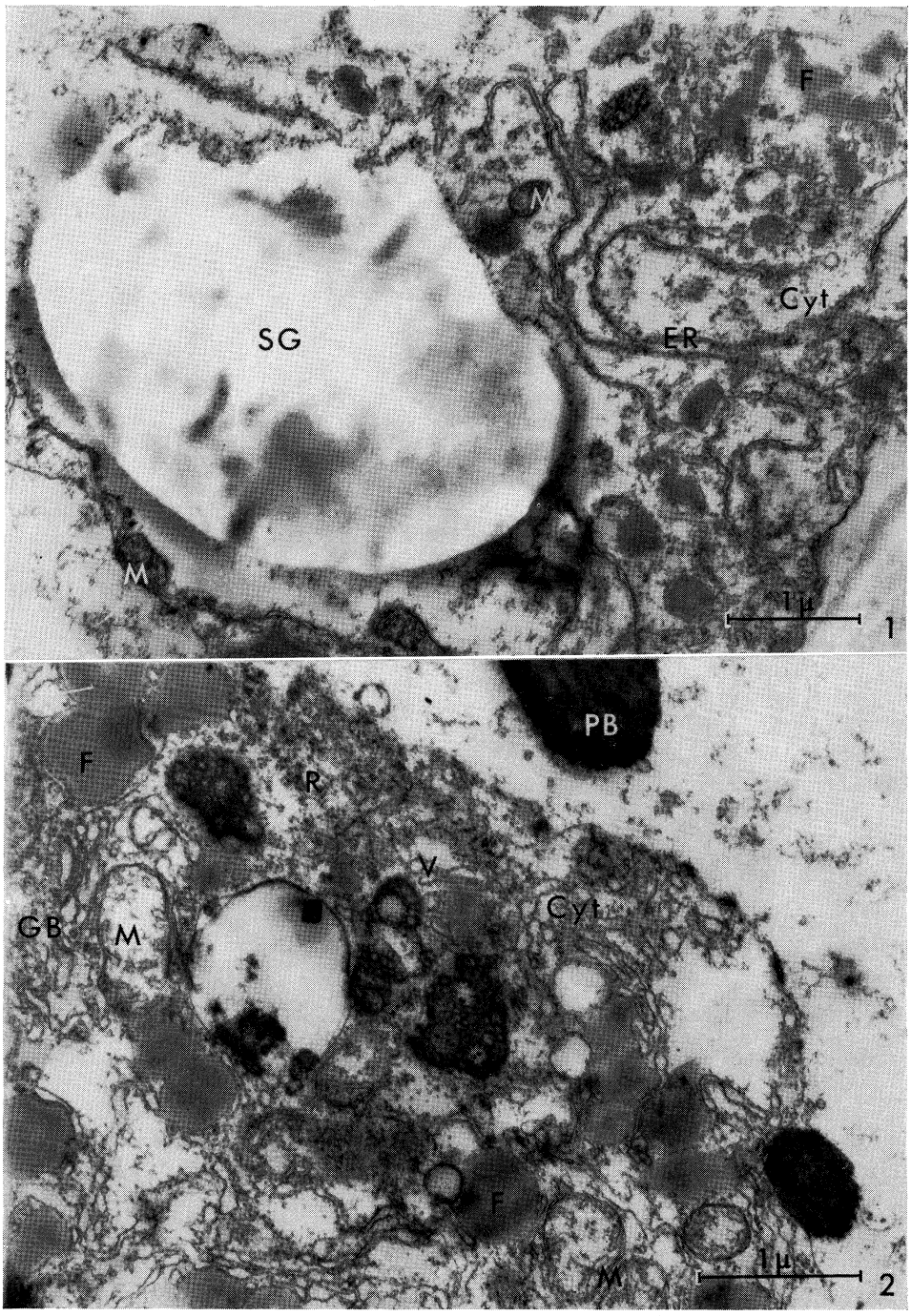
PEA COTYLEDONS DURING AND AFTER GERMINATION



PEA COTYLEDONS DURING AND AFTER GERMINATION



PEA COTYLEDONS DURING AND AFTER GERMINATION



PEA COTYLEDONS DURING AND AFTER GERMINATION