SUBCELLULAR ORGANIZATION OF THE DEVELOPING COTYLEDONS OF *PISUM SATIVUM* L.

By JOAN M. BAIN* and F. V. MERCER†

[*Manuscript received November* 15, 1965]

Summary

Morphological, anatomical, submicroscopical, and physiological changes in whole seeds and embryos of *Pisum sativum* L. cv. Victory Freezer were followed during 54 days of development of the seed. Four developmental phases—cell formation, cell expansion, synthesis of storage reserves, and maturation and dormancy—were recognized in the development of the embryo. Each phase was characterized by a distinctive physiology and a distinctive subcellular organization of the parenchyma cells. The subcellular organization associated with carbohydrate, protein, and fat metabolism and the significance of membranes in cell organization is described.

I. INTRODUCTION

Metabolic processes resulting in the formation and expansion of cells and the formation of storage substances, especially carbohydrate and protein, are very active in developing pea cotyledons, while the transition into dormancy is marked by a cessation of metabolism (Bisson and Jones 1932; Danielsson 1952; McKee, Robertson, and Lee 1955; McKee, Nestel, and Robertson 1955; Raacke 1957*a*, 1957*b*, 1957*c*; Turner and Turner 1957; Turner, Turner, and Lee 1957; Rowan and Turner 1957; Robertson *et al.* 1962). These organs, therefore, should be suitable material in which to relate subcellular organization and anabolic processes.

In the present study morphological, anatomical, and physiological changes in the whole seed and embryo of *Pisum sativum* L. cv. Victory Freezer were followed from fertilization to maturity (54 days). These changes were correlated with changes in the subcellular organization of the cotyledon cells from day 7 to day 45 of development. As the cultivar Victory Freezer differed from those used previously, it was necessary to make a number of anatomical, morphological, and physiological observations on this variety as a basis of reference against the existing data on the development of the pea seed. These observations are reported more fully elsewhere (Bain 1964).

II. MATERIALS AND METHODS

(*a*) *Source of Material*

Flowers of *P. sativum* cv. Victory Freezer growing in the Botany Garden, University of Sydney, were tagged at full blossom in early September. Pollination occurs approximately 24 hr before full blossom and fertilization is complete in the

* 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.

fully opened flower (Cooper 1938). Samples were taken at intervals up to 54 days from fertilization, by which time the pods were dry and the seeds dormant.

(*b*) *Preparation of Material for Microscopy*

(i) *Light Microscopy.*—Material was fixed in a solution of 1 part acetic acid to 3 parts of 70% ethanol, stored in 70% ethanol, and embedded in paraffin wax. Sections of 12 μ thickness were stained with Heidenhain's haematoxylin and orange G or safranin and fast green (Johansen 1940). Histochemical tests (iodine–potassium iodide for starch, mercuric bromphenol blue for protein, Sudan III for fat) were made on hand-sections of fresh or fixed material.

(ii) *Electron Microscopy.*—Whole seeds, whole embryos, or pieces of cotyledons were fixed up to the 18th–20th day; pieces of inner and outer cotyledon (1 mm^3) were fixed after the 22nd day. Tissue was fixed for 2 hr at approximately 5°C in buffered solutions of either 1% osmium tetroxide (Palade 1952) or 2% potassium permanganate (Luft 1956), made up according to the schedules given by Mercer and Birbeck (1961*a*) with sucrose added (Caulfield 1957). The washed material was stained with 2% uranyl acetate, dehydrated in an alcohol series, placed in propylene oxide, then in a mixture of propylene oxide and Araldite, and finally into an Araldite mixture which was based on that of Glauert and Glauert (1958). Some material was treated with 1% phosphotungstic acid in 70% alcohol during dehydration. The Araldite was polymerized at 70°C for 2 days; sections were cut with a diamond knife in a Sorvall "Porter-Blum" microtome and examined at 80 kV in a Siemens Elmiskop 1 electron microscope.

Osmium tetroxide gave better fixation than permanganate once the cells began to vacuolate. Starch grains were identified as clear areas in the plastids after both fixatives, and the protein reserves as membrane-bounded regions of dense contrast after osmium fixation (Plate 5, Figs. 1 and 2). These dense deposits were assumed to be protein since they increased in amount with increasing protein nitrogen of the tissue and reacted very strongly with the fixative. Varner and Schidlovsky (1963) have subsequently isolated similar material from pea cotyledons and identified it mainly as globulin. Irregular-shaped homogeneous regions (Plate 8, Fig. 1) after osmium fixation, which appeared similar to those described as lipid in electron micrographs of animal tissue, were classified as fat reserves, and assumed to correspond with the fat reserves identified histochemically.

(*c*) *Physiological Measurements*

(i) *Fresh Weight, Dry Weight, and Moisture Content.*—The fresh weight per seed was found at each sampling from the 10th to the 54th day, and that of the testa and the embryo separately from the 14th to the 39th day. Each sample was approximately 200 seeds up to the 20th day; and thereafter approximately 60 seeds. Dry weights of the testa and the embryo were found separately from the 17th to the 39th day, and those of the whole seed from the 14th to the 54th day, following drying for 24 hr at 80°C. These samples were then finely ground before analysis.

(ii) *Nitrogen Content.*—Total and protein nitrogen were determined by the method of McKenzie and Wallace (1954), protein nitrogen being determined after extraction with 75% ethanol (Turner 1949). Soluble nitrogen was recorded as their difference. Total protein was estimated as 6·25 times the protein nitrogen content. Data were found as milligrams per gram dry weight and expressed per embryo, testa, and seed.

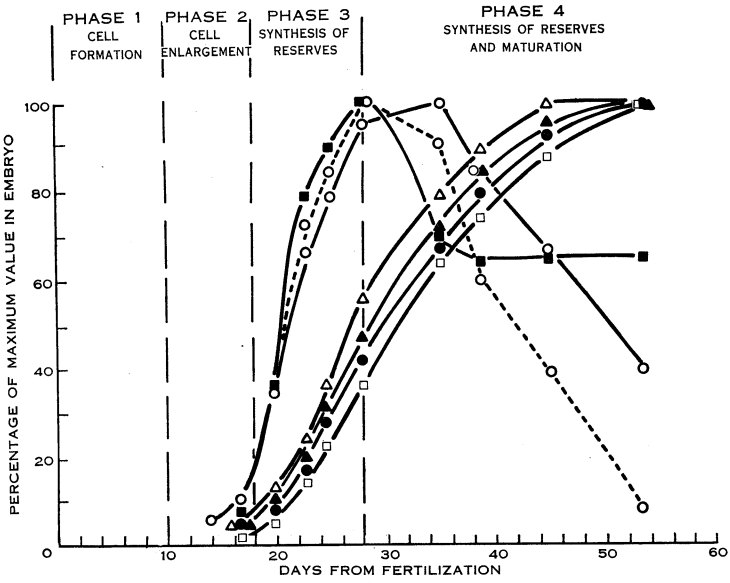


Fig. 1.—Development of the pea embryo divided into four phases based on the development of the cotyledon cells and characterized by distinct rates of growth and physiological change. Data for changes in fresh weight (○——○), dry weight (●——●), moisture content (○- - -○), total sugar content (■——■), starch content (□——□), total nitrogen content (△——△), and protein nitrogen content (▲——▲) are expressed as a percentage of their maximum value in the embryo. Maximum values are as follows: fresh weight 0·53 g; dry weight 0·23 g; moisture content 0·39 g; starch 65·4 mg; sugar 37·1 mg; total nitrogen 9·3 mg; protein nitrogen 9·1 mg.

(iii) *Carbohydrate Content.*—Starch was estimated by a method after Nielsen (1943). Total sugars and reducing sugars were determined according to Somogyi (1952). Non-reducing sugars were recorded as their difference. Data were found as milligrams per gram dry weight and expressed per embryo, testa, and seed.

III. Results

The morphological and anatomical data on the seeds and cotyledons are illustrated in Plates 1–4 and the physiological data (fresh weight, dry weight, moisture content, total and protein nitrogen, soluble carbohydrate and starch) are summarized in Figures 1–4. In general these data are similar to those described for other varieties of peas (see Introduction) although the previous observations for any one variety are mostly limited to the period from the 15th to the 38th day of

development of the whole seed and usually do not include simultaneous morphological, anatomical, and physiological observations for the one variety. The similarity of the various data for the 15th to the 38th day of development suggests that the observations for the cultivar Victory Freezer over the period 0–54 days may be taken as representative of all varieties (and vice versa). No previous data included observations on the ultrastructure of the cotyledon cells during development.

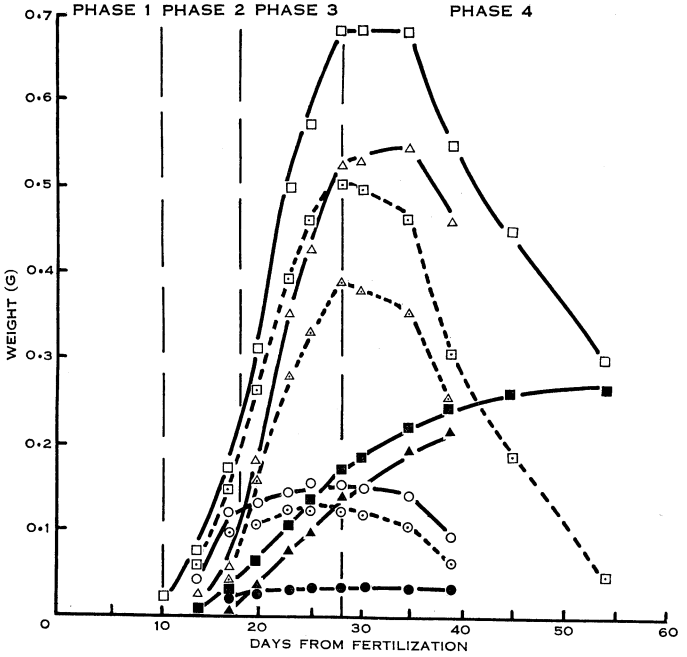


Fig. 2.—Changes in the fresh weight (□, △, ○), dry weight (■, ▲, ●), and moisture content (⊡, ◬, ⊙) respectively in the seed, embryo, and testa during the four phases based on the development of the pea embryo.

An examination of the data for Victory Freezer shows that four phases can be identified in the development of the cotyledons (Fig. 1). These include the phase of cell formation (from fertilization to the 10th day), the phase of cell expansion (from the 10th to the 18th–20th day), the phase of synthesis of storage products (18th–28th day), and the phase of maturation leading to dormancy (28th–54th day).

Since the bulk of the cotyledons consisted of parenchyma cells (Plates 2 and 3) these cells must have been responsible for the developmental phases of the cotyledons. Each phase was dominated by particular aspects of metabolism (Fig. 1). As described below the ultrastructure of the cells changed during each phase and it was possible to correlate ultrastructural changes with certain metabolic processes.

(*a*) *Phase 1—Cell Formation*

This extended from fertilization to about the 10th day. During this time cell division was very active. The embryo was still undifferentiated by the 7th day; the ultrastructure of the cells (Plate 6, Fig. 1) was similar to that of cells in other

meristematic tissue [e.g. stem apex (Buvat 1958), root apex (Whaley, Mollenhauer, and Leech 1960), and legume root nodules (Dart and Mercer 1965)]. The embryo was surrounded by disorganized nuclei and mitochondria, presumably part of the liquid endosperm (Plate 6, Fig. 2).

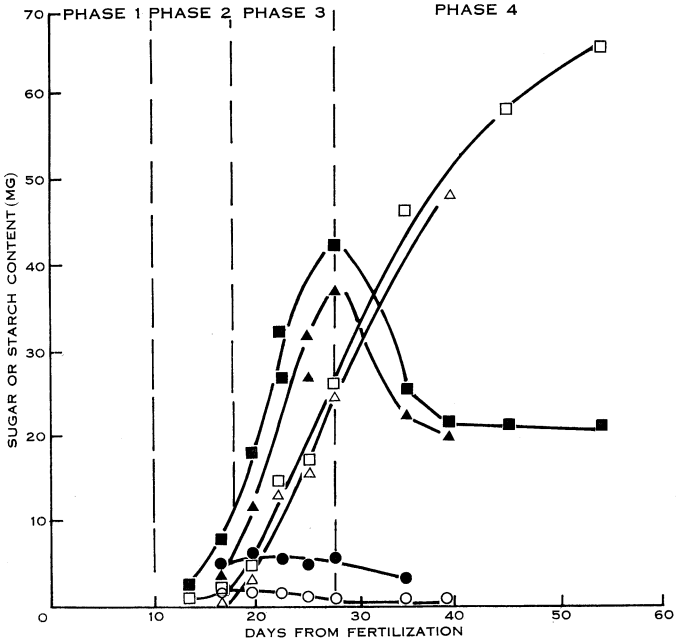


Fig. 3.—Changes in the sugar content (■, ▲, ●) and starch content (□, △, ○) respectively in the seed, embryo, and testa during the four phases based on the development of the pea embryo.

The embryo was differentiated by the 10th day, the majority of the cells were beginning to enlarge, and to lose their meristematic appearance (Plate 2, Fig. 2); cotyledon tissue could now be fixed satisfactorily in either osmium tetroxide or potassium permanganate, although the appearance of the cotyledon cells was very different in the two fixatives (Plate 6, Figs. 3 and 4). The cells had a large resting nucleus containing ribosome-like particles in the nucleoplasm; these were often clumped. The cytoplasm contained sparsely distributed ribosomes, and differentiated, but immature, mitochondria and plastids. The plastids were elliptical, about 3 μ in length, and contained a few parallel lamellae and poorly orientated grana. Occasional plastids contained a single, very small starch grain. After osmium fixation (Plate 6, Fig. 3) the endoplasmic reticulum was seen as a net work of small vesicles and the membranes were smooth. Very small vesicles, possibly submicroscopic vacuoles, were scattered through a granular cytoplasmic matrix. In contrast after permanganate fixation the membranes appeared parallel (Plate 6, Fig. 4). Electron-dense material was present in the loops of the Golgi bodies and in the adjacent cytoplasm, suggesting that the dense material was secreted by the bodies (Plate 6, Fig. 4; Plate 10, Fig. 1). Similar observations have been made by Whaley, Kephart, and Mollenhauer (1959) on Golgi bodies in the root tip cells of *Zea mays*.

Thus, at the end of phase 1, the cotyledon cells had a full complement of organelles and cell structures, but the vacuolar system was still developing.

Owing to the small size of the seed and cotyledons information about the composition (e.g. dry weight, carbohydrate, and nitrogen content) of the cotyledons during phase 1 was not obtained.

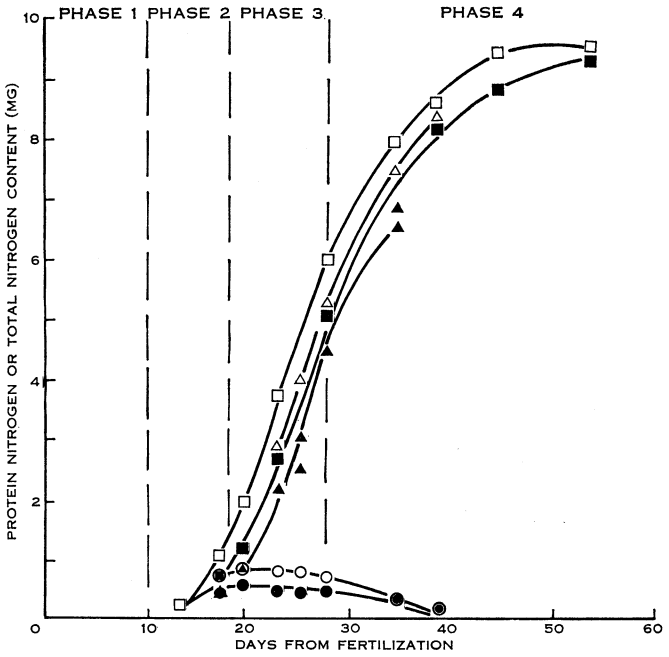


Fig. 4.—Changes in protein nitrogen content (■, ▲, ●) and total nitrogen content (□, △, ○) respectively in the seed, embryo, and testa during the four phases based on the development of the pea embryo.

(*b*) *Phase 2—Cell Expansion*

At the end of phase 1 the embryo occupied only a small part of the seed which consisted mostly of endosperm and testa (Plate 1). During phase 2 the embryo enlarged to fill the seed cavity; some liquid endosperm still exuded on cutting. Most of the increase in size was due to the expansion of the parenchyma cells of the cotyledons, whose volume increased approximately fourfold (Plate 2, Figs. 1–4).

Early in phase 2 the nuclei enlarged slightly, becoming lobed. These changes coincided with the transition from the meristematic to the expanding state. The nucleoli also became more conspicuous; the number of ribosome-like particles increased and became more uniformly dispersed through the nucleoplasm than previously. Presumably, these changes indicated a renewal of nuclear activity. As phase 2 proceeded the nuclei retained their lobed appearance, the nucleolar material remained prominent; the free ribosomes of the cytoplasm gradually increased and were very numerous by the 18th day (Plate 7, Fig. 1).

Extensive vacuolation of the cytoplasm occurred, this was apparent at both the microscopic (Plate 2, Figs. 2 and 3) and ultramicroscopic levels (Plate 7, Figs. 1 and 2). About the 10th day small vacuoles were found in the cytoplasm (Plate 6, Fig. 3). As time progressed these coalesced so that each cell contained several large vacuoles by the end of phase 2. This differentiation and expansion of the vacuolar system coincided with the expansion of the cells. The long axis of the plastids increased from about 3 μ to 8 μ during phase 2. The endoplasmic reticulum became more extensive and by the 19th day had formed an elaborate network of vesicles and cisternae throughout the cytoplasm. Since the total amount of cytoplasm, endoplasmic reticulum, and organelle volumes per cell also increased during phase 2, part of the increase in cell volume was due to the increase in protoplasm. Golgi bodies were prominent throughout phase 2 and, since their numbers per section did not change, possibly increased in number per cell as the cells expanded. Electron-dense material was present in their loops and adjacent cytoplasm, but in decreasing amounts as the phase progressed, suggesting that the activity of the bodies gradually became less during phase 2 (Plate 10, Fig. 2).

Storage products began to appear in the cells towards the end of phase 2, but the amount was insufficient to identify the cotyledon as a storage organ (Plate 2, Figs. 3 and 4). Small starch grains occurred in the stroma of some plastids as early as the 10th day, but most plastids contained a single starch grain by the 19th day. The chloroplasts were between 5 and 8 μ in length. Since the plastids increased in size more rapidly than the starch grains, their shapes and structures were not altered by the enlargement of the starch grains. By day 16 the tissue gave a positive iodine–potassium iodide reaction and starch was detected analytically (Figs. 1 and 3). Small irregularly shaped deposits of fat were seen in the electron micrographs of the cytoplasm about the 16th–17th day, but the tissue gave only a very weak Sudan III reaction. By the 17th–18th day traces of storage protein appeared on the inner face of the limiting membranes of some of the cytoplasmic vacuoles (Plate 7, Fig. 1).

Since the volume of cytoplasm and organelles formed during phase 2 far exceeded the trace of storage protein, which began to form only at the end of the phase, the changes in the total nitrogen and protein nitrogen of the tissue (Figs. 1 and 4) were probably due to the growth of the cytoplasm and organelles. Water and soluble carbohydrate (sucrose) increased together during phase 2 (Figs. 1, 2, and 3) confirming observations for other varieties of peas (see Introduction). Possibly this correlation reflected the expansion of the vacuolar system, with the soluble sugars being localized in the vacuolar solution.

Thus, at the ultrastructural level the major features of the cells during phase 2 were the differentiation and expansion of the vacuolar system, elaboration of the endoplasmic reticulum, growth of the organelles, growth of the cytoplasm, and a large increase in the number of ribosomes. These were correlated at the anatomical level with the expansion of the cells.

(c) *Phase 3—Synthesis of Storage Reserves*

The transition from phase 2 to phase 3 occurred at the 18th–20th day and was marked by the onset of rapid synthesis of starch and reserve protein (Figs. 1, 3, and 4).

During phase 3, the seed and cotyledons increased in volume (Plate 3) but much more slowly than in phase 2; the cotyledons differentiated into an inner tissue of rounded closely packed cells and an outer, narrower, tissue of more elongated cells. As phase 3 progressed both tissues showed increasingly positive histochemical reactions for starch, protein, and fat. The storage reserves (starch grains and protein bodies) and fat deposits could be seen to enlarge during phase 3 under the light microscope (Plate 3, Figs. 1, 2, and 3; Plate 4, Figs. 1 and 2).

Fresh weight and dry weight of the seed, now mainly embryo, increased throughout (Figs. 1 and 2). The fresh weight reached a maximum at day 28. Sugar content, mainly sucrose, also increased to a maximum by the 28th day, when the embryo contained 87% of the total sugar of the seed (Figs. 1 and 3). Starch content increased approximately sevenfold and by the 28th day the embryo contained 98% of the starch of the seed. Total nitrogen, mainly protein nitrogen, increased approximately fivefold during the phase (Fig. 4).

These changes in carbohydrate, protein, and fat content of the cotyledons were accompanied by changes in the fine structure of the cells of both the inner and outer cotyledon tissues. The synthesis of starch grains (one per plastid) occurred more rapidly than the plastids enlarged, disrupting the internal structure of the plastids. Single starch grains, some up to 10 μ in length, filled most of the volume of the plastids by the end of phase 3, compressing the lamellae and stroma against the limiting membranes (Plate 9, Fig. 1); the mass of starch grains almost obscured the microscopic structure of the cells (Plate 4, Figs. 1 and 2). At no stage of their existence, were starch grains observed in contact with the plastid membranes. A few small plastids, however, did not form starch and their lamellar system remained intact. Possibly two types of plastids were present.

The protein material, which accumulated on the inner surfaces of the membranes of the cytoplasmic vacuoles following the increase in free ribosomes in the cytoplasm at the end of phase 2, increased rapidly up to the 24th or 25th day. Thereafter, this material did not appear to increase in amount and was found clumped on the membranes or as aggregates within the vacuoles.

After about the 24th–25th day, three different morphological forms of endoplasmic reticulum could be recognized in the cytoplasm. The first, the most extensive, consisted of smooth membranes enclosing electron-translucent spaces, and forming a network through the cytoplasm. This probably represented a further differentiation of the reticulum present in phase 2. The second form was associated with the accumulation of a reserve protein, an electron-dense material starting to accumulate within the space between the membranes from about the 24th or 25th day onwards. The third form of reticulum differentiated about the 26th day, i.e. several days after the onset of reserve protein synthesis, as groups of parallel and paired granular membranes (Plate 8, Fig. 2). The ribosomes of these membranes accounted for only a very small proportion of the total ribosomes of the cell.

The storage protein, which first appeared within the second form of endoplasmic reticulum about the 24th day, increased in amount during the remainder of phase 3 and into phase 4. The resulting masses of protein became very conspicuous, showed great variation in shape, and appeared different in the inner and outer tissue by the

28th day (Plate 8, Figs. 3 and 4). It is proposed to refer to the reserve protein of the endoplasmic reticulum as "protein bodies" and that of the cytoplasmic vacuoles as "vacuole–protein bodies". In the outer cells the protein bodies were mainly spherical, with the protein aggregated mainly on the membranes (Plate 8, Fig. 4). Those in the inner cells were more irregular in shape, with the protein either completely filling the space between the membranes or forming irregularly shaped deposits within the spaces (Plate 8, Fig. 3). Sometimes the protein was enclosed by parallel membranes that opened into elliptical or spherical vesicles, which were completely or partly filled with protein (Plate 5, Fig. 1; Plate 8, Fig. 3). Since the rapid increase in nitrogen of the seed (Figs. 1 and 4) approximately paralleled the rapid enlargement of the protein bodies, most of the gain in total and protein nitrogen of the seed was probably due to the synthesis of storage protein rather than to an increase in total protoplasm.

Early in phase 3 most Golgi bodies did not contain any deposits of dense material although occasional deposits were associated with a few of them in the cytoplasm (Plate 10, Fig. 3). The size and amount of these deposits were far less than those observed earlier. Later in phase 3 the bodies usually appeared disorganized, and the bent swollen disks did not contain any dense material, with only a few small deposits being present in the surrounding cytoplasm (Plate 10, Fig. 4). These features suggest that the secretory function of the Golgi bodies gradually ceased in phase 3, at a time when the synthesis of storage protein was most active.

Irregularly shaped deposits of fat increased in amount in the cytoplasm (Plate 8, Fig. 1). By day 28 fat was also present in the cytoplasm as small Y-shaped bodies which appeared very distinct after uranyl acetate staining, but less obvious after phosphotungstic acid staining (Plate 8, Figs. 1 and 2).

Although most of the parenchyma cells had the type of organization described above, a few cells showed a different pattern of differentiation during phase 3. In these cells the protoplast had broken down and storage products, fragmented plastids, and mitochondria were dispersed through a vesiculated cytoplasm (Plate 9, Fig. 2). The role of these cells is not known, but as they persisted through dormancy and germination (Bain and Mercer 1966), they may be concerned with the transport of materials through the bulky cotyledon tissue which has a poorly developed vacuolar system.

Phase 3, as shown by the photomicrographs, electron micrographs, and the analytical data (Fig. 1), was characterized by the synthesis of storage products—starch, reserve protein, and fat.

(*d*) *Phase 4—Maturation–Dormancy*

At about the 28th day, a marked fall off occurred in the rate of increase in fresh weight, marking the transition into phase 4 (Figs. 1 and 2). Both seed and embryo continued to enlarge until about the 40th day, but only very slowly. After this time, the fresh weight decreased rapidly as the seeds passed into dormancy. The dry weight, however, continued to increase until about the 50th day, even though the cells were drying out. Histochemical tests and the analytical data (Fig. 1) showed that much of the increase in dry weight was due to increases in starch, protein, and fat. Light microscope observations showed that the protein bodies and starch grains increased in size, confirming the trend shown by the analytical data (Plate 4, Figs. 3 and 4).

Total sugar, mainly sucrose, decreased sharply between the 28th and 35th day (Figs. 1 and 3), thereafter remaining practically unchanged until the 54th day. The onset of loss of sugar, almost entirely from the cotyledons, coincided with the beginning of water loss from the seed and embryo. Starch per seed doubled (from 23 to 46 mg) between the 28th day and the 35th day, synthesis occurring at the same rate as in phase 3; it then increased to 65·5 mg per seed by the 54th day, but at a decreasing rate. Changes in total and protein nitrogen continued parallel, though at decreasing rates, throughout phase 4 (Fig. 4). Both increased in amount until the 54th day. Most of the total and protein nitrogen of the seed was in the cotyledons.

Considerable change in the ultrastructure of the cells due to the continued synthesis of storage products and the transition into dormancy occurred during phase 4.

Starch grains continued to enlarge up to the 45th day, disrupting the plastid structure yet further. By about the 35th day the stroma and lamellae had disappeared, leaving an electron-translucent zone between the starch grain and the limiting membranes. The plastid membranes were more difficult to resolve with further water loss and gradually disappeared, leaving the starch grains enclosed by the translucent zones. Fat deposits increased in size, possibly through the coalescence of the Y-shaped deposits which were so prevalent in the cytoplasm at the end of phase 3. The deposits varied in size and were scattered through the cytoplasm, but the smallest were concentrated adjacent to the cell walls. The endoplasmic reticulum fragmented and mostly disappeared as the cells dried out, leaving only a very few small vesicles scattered through the ground cytoplasm at about the 40th day. The ribosomes faded and by the 45th day could not be recognized in the cytoplasm. After the 45th day, all that could be resolved within the cells was a granular matrix containing masses of protein, starch, and fat, a few scattered vesicles, poorly differentiated mitochondria, and a vague round nucleus (Plate 9, Fig. 3).

IV. Discussion

The similarity between the physiological data for the cultivar Victory Freezer and other varieties described in the literature suggests that the developmental pattern of the cotyledon cells is similar for all varieties of peas. Some of the most striking features of the developmental pattern of the cotyledon cells are associated with the function of the cotyledon as a storage organ and several major structure–function systems can be described. These include the carbohydrate, protein, and fat systems which are considered in more detail below.

(*a*) *Subcellular Organization and Carbohydrate Metabolism*

The correlation between sugar content and water content of cotyledons during phases 2 and 3, which is similar to that observed for other varieties of peas (see Introduction), is closely associated in time with the differentiation and enlargement of the vacuolar system and the expansion of the cells. It is possible, therefore, that the sugars entering the cell during this time are largely accumulated into the vacuoles. Such a situation, by controlling the osmotic pressure of the vacuoles, could provide the positive turgor necessary for expansion of the cells.

If this interpretation is correct, it follows that the carbon skeletons for the synthesis of starch and reserve protein arise from carbohydrate being translocated into the cotyledon cells and not from the sugar already localized in the vacuoles. That is during phases 2 and 3 the vacuoles, plastids, and reserve protein bodies are sinks for carbohydrate entering the cell.

However, a different situation exists in phase 4, when the sugar content falls and the increase in starch from days 28 to 37 can be accounted for mainly by the decrease in sucrose (Fig. 3). This relation is consistent with the vacuolar sugars becoming available for starch synthesis. It is interesting that the vacuolar membranes become difficult to resolve in the electron micrographs after about the 30th day. Possibly the structure of the membranes alter as the cells lose water, allowing vacuolar sugars to leak into the cytoplasm.

The situation during approaching dormancy is more obscure since sucrose remains constant despite the continued synthesis of protein and starch and the falling water content of the cells. Reserves continue to be synthesized even in the nearly dormant seed (Fig. 3).

Starch always occurs in the cotyledon cells as single, often very large, grains in the plastids, in contrast to the several grains present in plastids of meristematic cells of pea roots and in the integument of the pea ovule. There is no evidence that smaller grains are formed concurrently with the storage grain in plastids of pea cotyledons and extruded into the cytoplasm, as in cereal endosperm (Buttrose 1960, 1963*a*). Since some of the plastids never form starch and retain an organized structure, it is possible that two classes of plastid are present in the pea cotyledons, possibly leucoplasts and chloroplasts.

The biochemical pathway of starch synthesis has been studied extensively in peas (Danielsson 1956; Turner and Turner 1957; Turner, Turner, and Lee 1957; Rowan and Turner 1957; Robertson *et al.* 1962) and in other plant material (De Fekete, Leloir, and Cardini 1960; Leloir, De Fekete, and Cardini 1961; Whelan 1961; Pottinger and Oliver 1962; Akazawa, Minamikawa, and Murata 1964). Starch phosphorylase was at first assumed to be associated with starch synthesis, but more recently starch synthetase has been given this role. Little is known of the spatial separation of the enzyme systems *in vivo*. In pea cotyledons the limiting membranes of the plastids remain intact and are never in contact with the starch grains while the grains are being laid down. This pattern suggests that the plastid membrane is concerned with the transport of precursors into the plastid and that the final steps in synthesis are located at the starch–stroma interface.

(*b*) *Subcellular Organization and Protein Synthesis*

Comparison of the subcellular organization found in pea cotyledon cells actively synthesizing protein with that found in other plant cells actively synthesizing protein, e.g. wheat endosperm (Graham *et al.* 1962; Jennings, Morton, and Palk 1963; Buttrose 1963*a*, 1963*b*; Morton and Raison 1963), shows that very marked differences occur at the structural level, even though a similar function is being performed. Graham *et al.* (1962) and Jennings, Morton, and Palk (1963) likened the ultrastructure of a wheat endosperm cell accumulating protein within membrane-bound regions of

a granular reticulum (internal secretion) to that of an animal-secreting cell. However, Morton and Raison (1963) suggested that protein synthesis and storage occurs within a specialized organelle (the proteoplast) in the wheat endosperm cells. This membrane-bound organelle, containing ribosomes embedded in a matrix, has been described as yet, only for triploid tissue and in the present work with diploid pea tissue no comparable organization was found.

Mercer and Birbeck (1961*b*) classified animal cells actively synthesizing protein into two classes, according to whether the protein is secreted (as in glands) or retained within the protoplast (as in mammalian epidermal cells, myoblasts, and erythroblasts); a distinctive ultrastructure was associated with each of these classes. The secretory cells were shown to have an elaborate network of long, parallel, granular membranes and protein was secreted into vesicles in the cytoplasm. In contrast, the retaining cell had no such arrangement of parallel membranes; the ribosomes were scattered through the cytoplasm, and the protein was not secreted in vesicles. Sjöstrand and Hanzon (1954); Dalton and Felix (1956); Farquhar and Wellings (1957); Hollmann (1959); Wellings and Deome (1961); and Warshawsky, Le Blond, and Droz (1963) have shown in studies of animal cells which secrete protein that protein is formed between the segments of the granular endoplasmic reticulum, transported to the Golgi body for condensation into granules within vesicles associated with this body, and discharged into the cytoplasm ready for secretion from the cell. Variation in the ultrastructural organization associated with protein synthesis increases as more types of tissue are studied. For example, crayfish oocytes have the endoplasmic reticulum of a secreting cell, but the protein material which is secreted into spaces between parallel, granular membranes is not condensed into granules for secretion within the Golgi body; instead, it is transported along unoriented cisternae of the endoplasmic reticulum and accumulated as large proteinaceous bodies in expanded cisternae regions (Beams and Kessel 1963).

By comparison with animal cells, pea cotyledon cells can be regarded as having the retaining type of cell structure during phases 1 and 2, when protoplasmic proteins are being synthesized. During phases 3 and 4, the cells function as secreting cells in that storage protein is secreted intracellularly in membrane-bound units. The cytoplasmic organization of these cells, however, still resembles that of the retaining cell, in that the endoplasmic reticulum is mainly a network of smooth membranes, and ribosomes are randomly scattered through the cells.

The secretion of storage protein in the pea cell most closely resembles the formation of yolk in the oocytes of the crayfish (Beams and Kessel 1963), except that the endoplasmic reticulum in pea is smooth, not granular as in the oocyte.

The storage protein in peas consists of an albumin fraction which is present in greatest amount in young seeds and a globulin fraction (vicilin and legumin) which is present in greatest amount in older seeds (Danielsson 1952). These fractions have not been identified in the electron micrographs, although from Danielsson's data, the albumin could be the protein that appears early in phase 3 in the large vacuoles, and the globulin the protein that appears later in phase 3 in the endoplasmic reticulation. Alternatively as suggested by Buttrose (1963*a*) for the proteins of wheat endosperm, all protein fractions may occur together.

Neither the isolated patches of granular endoplasmic reticulum, which appeared late in phase 3 (Plate 8, Fig. 2) and which resembled the granular reticulum of the animal secreting cell, nor the Golgi bodies appeared to be involved in the synthesis of storage protein in peas because their maximum development did not coincide with the period of protein synthesis. The distribution of a dense material between the loops of the Golgi bodies and in the cytoplasm, particularly in phase 1 and 2 indicates, however, that the Golgi body performs some type of secretory function.

TABLE 1

MEMBRANES IN THE MATURE PEA COTYLEDON CELL CLASSIFIED ACCORDING TO THEIR STRUCTURAL POSITION IN THE SUBCELLULAR ORGANIZATION

Type of Membrane	Structural Position in the Cell
Plasmalemma	Encloses protoplast
Tonoplast	Encloses cytoplasmic vacuoles containing sugars
Mitochondrial	Encloses mitochondrion
Plastid	Encloses leuco-chloroplast
Nuclear	Encloses nucleus
Golgi	Encloses disks of the Golgi body
Smooth endoplasmic reticulum—type *a*	Encloses empty cisternae and vesicles (phases 3 and 4)
Smooth endoplasmic reticulum—type *b*	Encloses storage protein (phases 3 and 4)
Granular endoplasmic reticulum	Encloses parallel empty spaces (phase 3)
Cytoplasmic membranes—type *a*	Enclose large vacuoles in which storage protein is secreted in phases 2 and 3
Cytoplasmic membranes—type *b*	Enclose fat reserves (phases 3 and 4)

Electron micrographs indicate that two populations or groups of ribosomes occur in the cytoplasm. One group is formed while the cells are in the meristematic state, the other as phase 2 progresses, preceding the onset of starch and protein synthesis. Possibly, the ribosomes formed in phase 1 are concerned with the synthesis of the enzymic equipment of the meristematic-enlarging cell, while those formed in phase 2 are concerned with the synthesis of storage protein and the synthesis of the enzymes of the protein and starch systems. If plastids are self-duplicating organelles, however, the enzymes of starch synthesis may be independent of the ribosomes of the cytoplasm. Should ribosomes be the sites of protein synthesis in peas, protein molecules must be transported considerable distances through the cytoplasm to the membranes for secretion, unless the ribosomes contact the membranes during cyclosis and give up the protein directly.

If ribosomes are manufactured in the nucleus, it is of interest that the renewal of nuclear and nucleoli activity in phase 2 preceded the build up of ribosomes in phase 2, and the build up of ribosomes preceded the synthesis of reserve protein.

(*c*) *Subcellular Organization and Fat Synthesis*

No clear relation between structure and fat synthesis can be deduced from the electron micrographs. Fat synthesis does not appear to be associated with an organelle-type of structure, although some fat deposits appear to be enclosed by a

membrane. The only structural correlation recorded is that of the disappearance of membranes and the rapid build up of fat deposits as the cells dry out during phase 4.

(*d*) *Membranes and Subcellular Organization*

Membranes are conspicuous features of the ultrastructure of the cotyledon cells, and on the basis of their structural position within the cell, 11 different membranes can be recognized (Table 1). As these membranes of differing structural position enclose regions or compartments of the protoplast having different physiological activities, it seems possible that the membranes may have distinct physiological properties. Membranes, therefore, appear to have a central role in the subcellular organization of the cotyledon cells. They may control metabolism in several ways: by passively separating functionally different parts of the cell; by actively controlling the movement of metabolites between the different regions; or by possessing specific groupings of enzymes, and, therefore, having specific metabolic properties.

V. Acknowledgments

The authors wish to acknowledge the assistance of Miss Rosemary Mullens and Miss Barbara Williams in sectioning the material for electron microscopy; also that given by Mr. J. Smydzuk in making the analytical determinations on prepared samples, and Mr. P. R. Maguire for the photographs reproduced in Plate 1.

VI. References

Akazawa, T., Minamikawa, T., and Murata, T. (1964).—Enzymic mechanism of starch synthesis in ripening rice grains. *Plant Physiol.* **39**: 371–8.

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. (1966).—Subcellular organization of the cotyledons in germinating seeds and seedlings of *Pisum sativum* L. *Aust. J. Biol. Sci.* **19**: 69–84.

Beams, H. W., and Kessel, R. G. (1963).—Electron microscope studies on developing crayfish oocytes with special reference to the origin of yolk. *J. Cell Biol.* **18**: 621–49.

Bisson, C. S., and Jones, H. A. (1932).—Changes accompanying fruit development in the garden pea. *Plant Physiol.* **7**: 91–106.

Buttrose, M. S. (1960).—Submicroscopic development and structure of starch grains in cereal endosperm. *J. Ultrastruct. Res.* **4**: 231–57.

Buttrose, M. S. (1963*a*).—Ultrastructure of the developing wheat endosperm. *Aust. J. Biol. Sci.* **16**: 305–17.

Buttrose, M. S. (1963*b*).—Ultrastructure of the developing aleurone cells of wheat grain. *Aust. J. Biol. Sci.* **16**: 768–74.

Buvat, R. (1958).—Recherches sur les infrastructures du cytoplasma dans les cellules du méristème apical des ébauches foliares et de feuilles développées d'*Elodea canadensis*. *Ann. Sci. Nat. Bot.* **18**: 121–61.

Caulfield, J. B. (1957).—Effects of varying the vehicle for OsO_4 in tissue fixation. *J. Biophys. Biochem. Cytol.* **3**: 827–9.

Cooper, D. C. (1938).—Embryology of *Pisum sativum*. *Bot. Gaz.* **100**: 123–32.

Dalton, A. J., and Felix, M. D. (1956).—A comparative study of the Golgi complex. *J. Biophys. Cytol.* **2** (suppl.): 79–84.

Danielsson, C. E. (1952).—A contribution to the study of the synthesis of reserve proteins in ripening pea seeds. *Acta Chem. Scand.* **6**: 149–59.

Danielsson, C. E. (1956).—Starch formation in ripening pea seeds. *Physiol. Plant.* **9**: 212–19.

DART, P., and MERCER, F. V. (1965).—Observations on the fine structure of the meristem of root nodules from some annual legumes. *J. Linn. Soc. N.S.W.* **90** (3): (in press).

DE FEKETE, M. A. R., LELOIR, L. F., and CARDINI, C. E. (1960).—Mechanism of starch biosynthesis. *Nature, Lond.* **187**: 918–19.

FARQUHAR, M. C., and WELLINGS, S. R. (1957).—Electron microscopic evidence suggesting secretory granule formation within the Golgi apparatus. *J. Biophys. Biochem. Cytol.* **3**: 319–22.

GLAUERT, A. M., and GLAUERT, R. H. (1958).—Araldite as an embedding medium for electron microscopy. *J. Biophys. Biochem. Cytol.* **4**: 191–4.

GRAHAM, J. S. D., JENNINGS, A. C., MORTON, R. K., PALK, B. A., and RAISON, J. K. (1962).—Protein bodies and protein synthesis in developing wheat endosperm. *Nature, Lond.* **196**: 967–9.

HOLLMAN, K. H. (1959).—L'ultrastructure de la glande mammaire normale de la souris en lactation. *J. Ultrastruct. Res.* **2**: 423–43.

JENNINGS, A. C., MORTON, R. K., and PALK, B. A. (1963).—Cytological studies of protein bodies of developing wheat endosperm. *Aust. J. Biol. Sci.* **16**: 366–74.

JOHANSEN, D. A. (1940).—"Plant Microtechnique." (McGraw-Hill Book Co. Inc.: New York.)

LELOIR, L. F., DE FEKETE, M. A. R., and CARDINI, C. E. (1961).—Starch and oligosaccharide synthesis from uridine diphosphate glucose. *J. Biol. Chem.* **236**: 636–41.

LUFT, J. H. (1956).—Permanganate—a new fixative for electron microscopy. *J. Biophys. Biochem. Cytol.* **2**: 799–801.

MCKEE, H. S., NESTEL, L., and ROBERTSON, R. N. (1955).—Physiology of pea-fruits. II. Soluble nitrogenous constituents in the developing fruit. *Aust. J. Biol. Sci.* **8**: 467–75.

MCKEE, H. S., ROBERTSON, R. N., and LEE, J. B. (1955).—Physiology of pea fruits. I. The developing fruit. *Aust. J. Biol. Sci.* **8**: 137–63.

MCKENZIE, H. A., and WALLACE, H. S. (1954).—The Kjeldahl determination of nitrogen. A critical study of digestion conditions—temperature, catalyst, and oxidizing agent. *Aust. J. Chem.* **7**: 55–70.

MERCER, E. H., and BIRBECK, M. S. C. (1961*a*).—"Electron Microscopy: a Handbook for Biologists." (Blackwell Scientific Publications, Ltd.: Oxford.)

MERCER, E. H., and BIRBECK, M. S. C. (1961*b*).—Cytology of cells which synthesize protein. *Nature, Lond.* **189**: 558–60.

MORTON, R. K., and RAISON, J. K. (1963).—A complete intracellular unit for incorporation of amino-acid into storage protein utilizing adenosine triphosphate generated from phytate. *Nature, Lond.* **200**: 429–33.

NIELSEN, J. P. (1943).—Rapid determination of starch. *Industr. Engng. Chem. (Analyt. Ed.).* **15**: 176–9.

PALADE, G. E. (1952).—A study of fixation for electron microscopy. *J. Exp. Med.* **95**: 285–98.

POTTINGER, P. K., and OLIVER, I. T. (1962).—The intracellular distribution of uridine diphosphate glucose starch synthetase in potato tubers. *Biochim. Biophys. Acta* **58**: 303–6.

RAACKE, I. D. (1957*a*).—Protein synthesis in ripening pea seeds. I. Analysis of whole seeds. *Biochem. J.* **66**: 101–10.

RAACKE, I. D. (1957*b*).—Protein synthesis in ripening pea seeds. II. Development of embryo and seed coats. *Biochem. J.* **66**: 110–13.

RAACKE, I. D. (1957*c*).—Protein synthesis in ripening pea seeds. III. Protein synthesis. Study of pods. *Biochem. J.* **66**: 113–16.

ROBERTSON, R. N., HIGHKIN, H. R., SMYDZUK, J., and WENT, F. W. (1962).—The effect of environmental conditions on the development of pea seeds. *Aust. J. Biol. Sci.* **15**: 1–15.

ROWAN, K. S., and TURNER, D. H. (1957).—Physiology of pea fruits. V. Phosphate compounds in the developing seed. *Aust. J. Biol. Sci.* **10**: 414–25.

SJÖSTRAND, F. S., and HANZON, V. (1954).—Ultrastructure of Golgi apparatus of exocrine cells of mouse pancreas. *Exp. Cell Res.* **7**: 415–29.

SOMOGYI, M. (1952).—Notes on sugar determinations. *J. Biol. Chem.* **195**: 19–23.

TURNER, D. H., and TURNER, J. F. (1957).—Physiology of pea fruits. III. Changes in starch and starch phosphorylase in the developing seed. *Aust. J. Biol. Sci.* **10**: 302–9.

TURNER, J. F. (1949).—The metabolism of the apple during storage. *Aust. J. Sci. Res.* B **2**: 138–53.

TURNER, J. F., TURNER, D. H., and LEE, J. B. (1957).—Physiology of pea fruits. IV. Changes in sugars in the developing seed. *Aust. J. Biol. Sci.* **10**: 407–13.

VARNER, J. E., and SCHIDLOVSKY, G. (1963).—Intracellular distribution of proteins in pea cotyledons. *Plant Physiol.* **18**: 139–44.

WARSHAWSKY, H., LE BLOND, C. P., and DROZ, B. (1963).—Synthesis and migration of proteins in the cells of the exocrine pancreas as revealed by specific activity determination from radio-autographs. *J. Cell Biol.* **16**: 1–23.

WELLINGS, S. R., and DEOME, K. B. (1961).—Milk protein droplet formation in the Golgi apparatus of the C3H/Crgl mouse mammary gland. *J. Biophys. Biochem. Cytol.* **9**: 479–85.

WHALEY, W. G., KEPHART, J. E., and MOLLENHAUER, H. H. (1959).—Developmental changes in the Golgi apparatus of maize root cells. *Am. J. Bot.* **46**: 743–51.

WHALEY, W. G., MOLLENHAUER, H. H., LEECH, J. H. (1960).—The ultrastructure of the meristematic cell. *Am. J. Bot.* **47**: 401–50.

WHELAN, W. J. (1961).—Recent advances in the biochemistry of glycogen and starch. *Nature, Lond.* **190**: 954–7.

EXPLANATION OF PLATES 1–10

Plates 2, 3, and 4 are light micrographs of pea cotyledon cells. All figures in these plates, except Plate 4, Figure 3, are taken from fixed tissue which was embedded in paraffin wax, sectioned at 12 μ, and stained with Heidenhain's haematoxylin and orange G. Plate 4, Figure 3, is from hand-sectioned material stained with mercuric bromphenol blue. Plates 5–10 are electron micrographs of pea cotyledon tissue which was fixed at different times during development, "stained" with uranyl acetate, embedded in Araldite, and sectioned. All material with the exception of that in Plate 5, Figure 2, Plate 6, Figure 4, and Plate 10, Figure 1, was fixed in 1% buffered osmium tetroxide for 2 hr; the other material was fixed in 2% buffered potassium permanganate solution

PLATE 1

Growth of the seed and of the embryo of *Pisum sativum* L. cv. Victory Freezer during the four phases based on the development of the embryo.

PLATE 2

Fig. 1.—Longitudinal section through a 10-day-old embryo (at end of phase 1) showing the cotyledon (*C*) and part of the plant axis (*A*). Some areas of the cotyledon still appear meristematic and others are showing cell enlargement. × 138.

Fig. 2.—Longitudinal section through a 13-day-old embryo (early phase 2) showing the enlarging cells of the cotyledon tissue (*C*) and part of the developing plant axis (*A*). × 138.

Fig. 3.—Section through a cotyledon (*C*) after 19 days' development (end of phase 2) showing the epidermal cells (*E*), the small hypodermal cells (*H*) and the now enlarged cells of the underlying tissue. Starch grains and nuclei are prominent in these cells. ×138.

Fig. 4.—Appearance of starch grains (*SG*) in the cells of a 19-day-old cotyledon (end of phase 2). The nucleus (*N*) and nucleolus are prominent in these cells. ×550.

PLATE 3

Fig. 1.—Section through a 22-day-old cotyledon (early phase 3) showing the epidermis (*E*) and the outer cotyledon tissue (*OC*) which is separated from the inner cotyledon tissue by a vascular strand (*VS*). The cells of the outer cotyledon tissue tend to be elongated in the direction of the epidermis. All cells contain a prominent nucleus (*N*), increasing numbers of starch grains (SG), and small rounded bodies which were classed as storage protein reserves (*P*). ×138.

Fig. 2.—More detailed structure of cells as shown in Plate 3, Figure 1. Starch grains (*SG*) have increased in size (compare Plate 2, Fig. 4). The nucleolus (*Nuc*) is prominent in the nucleus (*N*). ×550.

Fig. 3.—Part of a 24-day-old cotyledon (early phase 3). Starch reserves (*SG*) now obscure the detail of the nucleus (*N*) and storage protein (*P*) in the cells. Cracks are evident in the starch grains. ×550.

Plate 4

Fig. 1.—Outer cotyledon tissue showing the appearance of cells after 28 days' development (end of phase 3). Starch grains (*SG*) are conspicuous in these cells which tend to be elongated at right angles to the epidermis (*E*) and have very small intercellular spaces. ×138.

Fig. 2.—Inner cotyledon tissue showing the appearance of cells after 28 days' development (end of phase 3). Starch grains (*SG*) are conspicuous and the cells are rounded, with large intercellular spaces (*IS*). ×138.

Fig. 3.—The 35-day-old cotyledon cells (phase 4) heavily stained blue with mercuric bromphenol blue, indicating the high protein content in the cells. Starch grains are visible as clear areas in the dense cell contents. ×138.

Fig. 4.—A cotyledon cell after 40 days' development. Starch grains (*SG*) are conspicuous in the dehydrated cell and the nucleus (*N*) is difficult to resolve. ×550.

Plate 5

Comparison of storage reserves after fixation in osmium tetroxide and potassium permanganate

Fig. 1.—Part of a cell at the end of 30 days' development (beginning of phase 4) showing the form of the storage material in the inner cotyledon tissue after fixation in buffered osmium tetroxide. Protein reserves or protein bodies (*PB*) are seen as dense osmiophilic regions within the network of the endoplasmic reticulum (*ER*). A starch grain (*SG*) appears as an electron-translucent body withdrawn from the limiting membrane of the plastid (*PLM*). Mitochondria (*M*) and the cell wall (*CW*) are also shown. ×10,000.

Fig. 2.—Shows the form of the storage materials after fixation with potassium permanganate. A starch grain (*SG*), appears as a clear area within the plastid. The limiting membrane of the plastid (*PLM*), and the stroma (*S*) and remains of lamellae (*L*) and grana (*G*) are very conspicuous after potassium permanganate. Protein reserves (*PB*) appear slightly denser than the cytoplasmic matrix and are enclosed by limiting membranes which are more obvious than in the osmium-fixed material. The endoplasmic reticulum (*ER*) is represented by paired membranes, but bears little resemblance to the appearance of the reticulum after osmium fixation (Fig. 1). Mitochondria (*M*) are also present. ×10,000.

Plate 6

Fig. 1.—The structure of cells from a 7-day-old cotyledon is typically that of a meristematic cell. The nucleus (*N*) occupies a large proportion of the cell volume and the nucleolus (*Nuc*) is very conspicuous. The dense cytoplasm (*Cyt*) contains numerous ribosomes, which are not attached to the membranes of the reticulum, and a few small vacuoles (*Vac*). The cell wall (*CW*) appears as a thin wavy band, which is typical of cells recently in division. ×7500.

Fig. 2.—The detailed structure of the endosperm surrounding the 7-day-embryo is shown in this figure. The endosperm, enclosed by a limiting membrane which is possibly analogous to the plasmalemma, has withdrawn away either from the surrounding embryo sac wall or from the embryo itself. It consists of a vesiculated cytoplasmic matrix (*Cyt*) in which many nuclei (*N*) and mitochondria (*M*) are embedded. The nuclei and mitochondria appear partly disorganized. ×7500.

Fig. 3.—Cotyledon cells are showing a high degree of vacuolation (*Vac*) by the 10th day (end of phase 1). The nucleus (*N*) and nucleolus (*Nuc*) are prominent in the cells and the cytoplasm (*Cyt*) is becoming a vesicular network in the now expanding cells. Ribosomes (*R*) are obvious in the cytoplasmic matrix. The cell wall (*CW*) has become thicker, but its outline is still wavy. ×7500.

Fig. 4.—Cell structure at the 10th day (end of phase 1) after fixation with potassium permanganate. The organelles are more obvious than in the osmium-fixed cells. Poorly developed grana (*G*) are seen in the plastids (*Pl*) which can therefore be regarded as chloroplasts. An elaborate system of paired membranes of the endoplasmic reticulum (*ER*) extends through the cytoplasm (*Cyt*). The mitochondria (*M*) are similar to other plant mitochondria. Deposits of electron-dense material are seen in the cytoplasm and these resemble the dense material present in the loops of the Golgi bodies (*GB*). The dense material appears to be secreted by the Golgi bodies. The cell wall (*CW*) is shown. ×15,000.

Plate 7

Fig. 1.—The number of ribosomes in the cytoplasm increased rapidly during phase 2, but the synthesis of storage protein did not commence until the end of the phase. In this figure, at the 18th–20th day (end of phase 2), the cytoplasm (*Cyt*) is packed with ribosomes (*R*) which are not attached to membranes. Traces of electron-dense material (*P*), later correlated with the increasing protein content of the seeds, are present at the interface of the cytoplasm and the large cytoplasmic vacuoles (*Vac*). At intervals a membrane can be identified at the vacuole–cytoplasm interface. A deposit of fat (*F*) is shown embedded in the cytoplasm. Plasmodesmata (*Pd*) are shown crossing through the cell wall (*CW*). ×40,000.

Fig. 2.—The protein material (*P*) which first appeared late in phase 2 increased markedly during phase 3. By the 24th day, this material is seen in large quantities on the limiting membranes of the large cytoplasmic vacuoles (*Vac*). Very small deposits of an electron-dense material are associated with the Golgi body (*GB*), but it has not been observed in direct contact with the accumulating protein reserves. Most of the loops of the Golgi disks and the vesicles which appear to be budded off from the body do not contain electron-dense material. The endoplasmic reticulum (*ER*) is an elaborate network through the cytoplasm (*Cyt*). Mitochondria (*M*), fat deposits (*F*) (occasionally separated from the cytoplasm by a membrane), and ribosomes (*R*) are embedded in the cytoplasmic matrix. ×30,000.

Plate 8

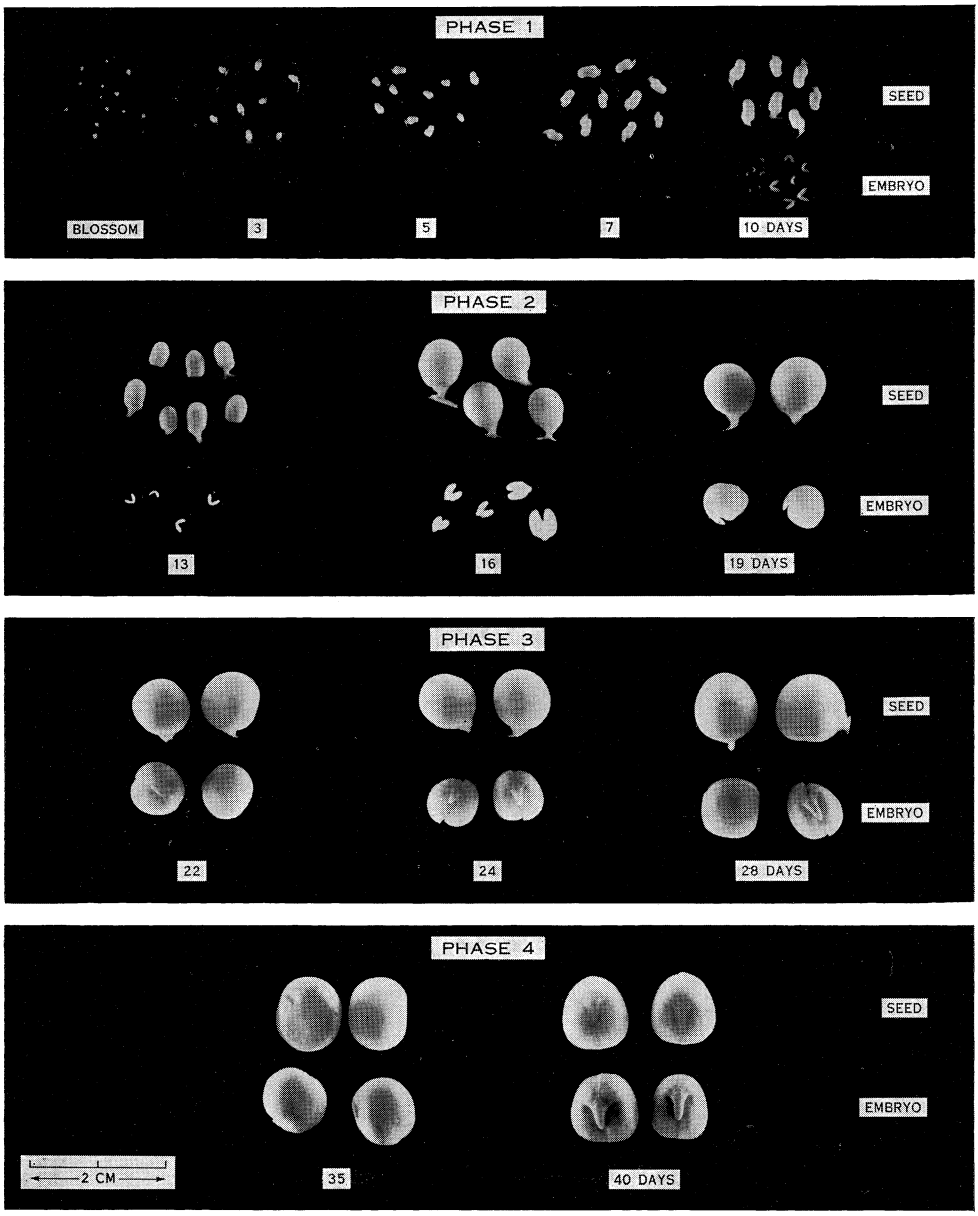
Fig. 1.—Represents the appearance of fat material (*F*) deposited in the cytoplasm in the maturing cotyledon by the end of phase 3. ×20,000.

Fig. 2.—Isolated groups of paired and parallel membranes developed in the cytoplasm about the 26th day (towards the end of phase 3). These membranes, though granular, did not appear to be associated with the formation of storage reserves. Ribosomes and Y-shaped bodies of fat material (*F*) are shown in the cytoplasm (*Cyt*). The cell wall (*CW*) is much thicker than previously. ×20,000.

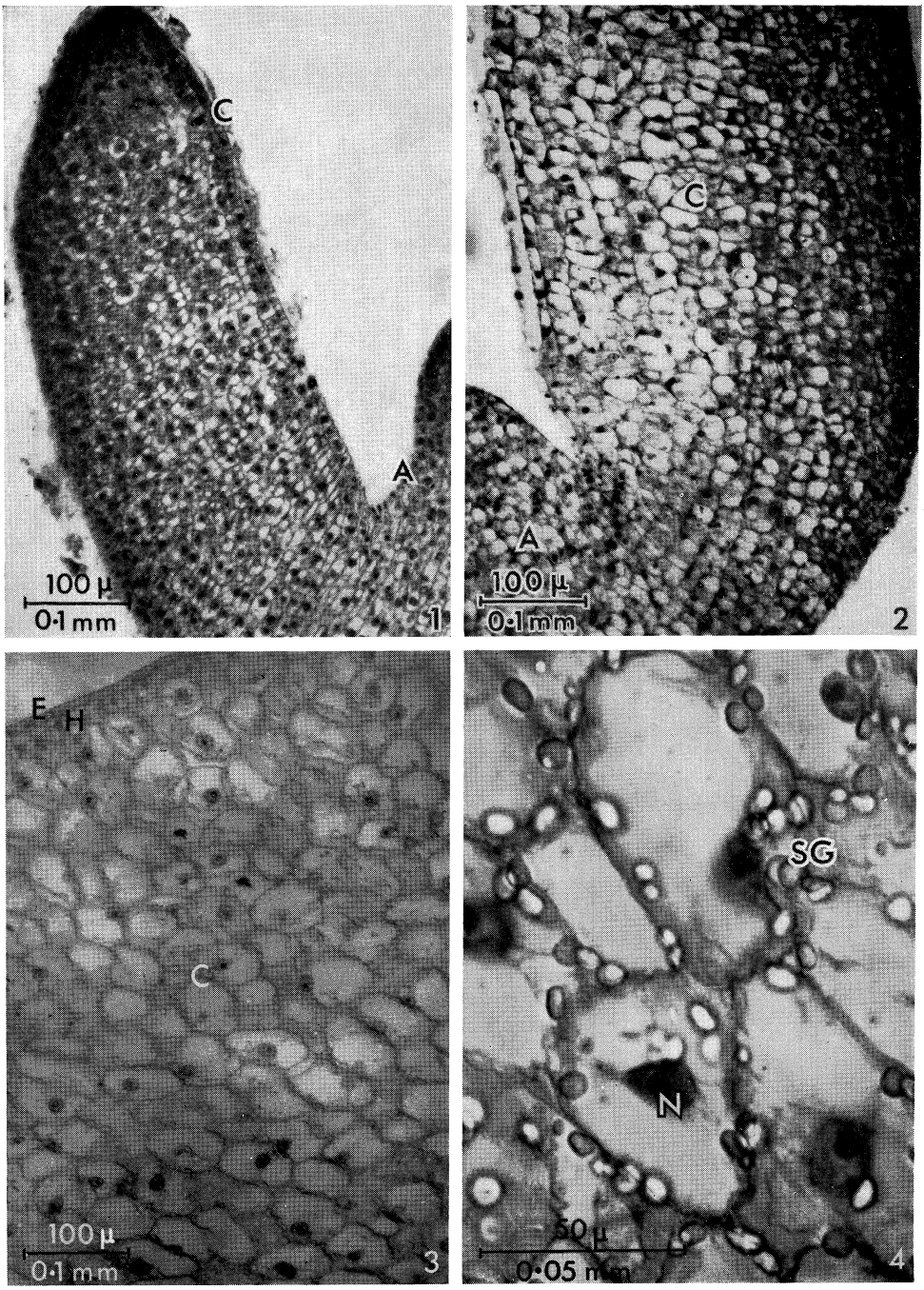
Fig. 3.—Two sites of accumulation of electron-dense material (protein) may be recognized in the cell by the end of phase 3. Protein material (*P*) is found in the large vacuoles (*Vac*) which formed in the cytoplasm during phase 2. Other protein material (*PB*) is found as irregular deposits between the spaces of the endoplasmic reticulum; in some instances it appears as though the protein could have been secreted between the parallel membranes and then accumulated in their enlarged ends. Disorganization of the membranes of the endoplasmic reticulum and of the Golgi body (*GB*) is now obvious. The difference in texture in the material of the protein body is associated with gradual loss of water from the tissue. ×15,000.

Fig. 4.—Similarly, in the outer cotyledon cell, protein (*P*) occurs in two sites, the protein bodies (*PB*) in the cytoplasm (*Cyt*) and in the large cell vacuoles (*Vac*). A starch grain (*SG*) is shown withdrawn from the remains of the plastid stroma (*S*) and lamellae (*L*) after 28 days' development (end of phase 3). ×12,500.

PEA COTYLEDONS DURING SEED DEVELOPMENT

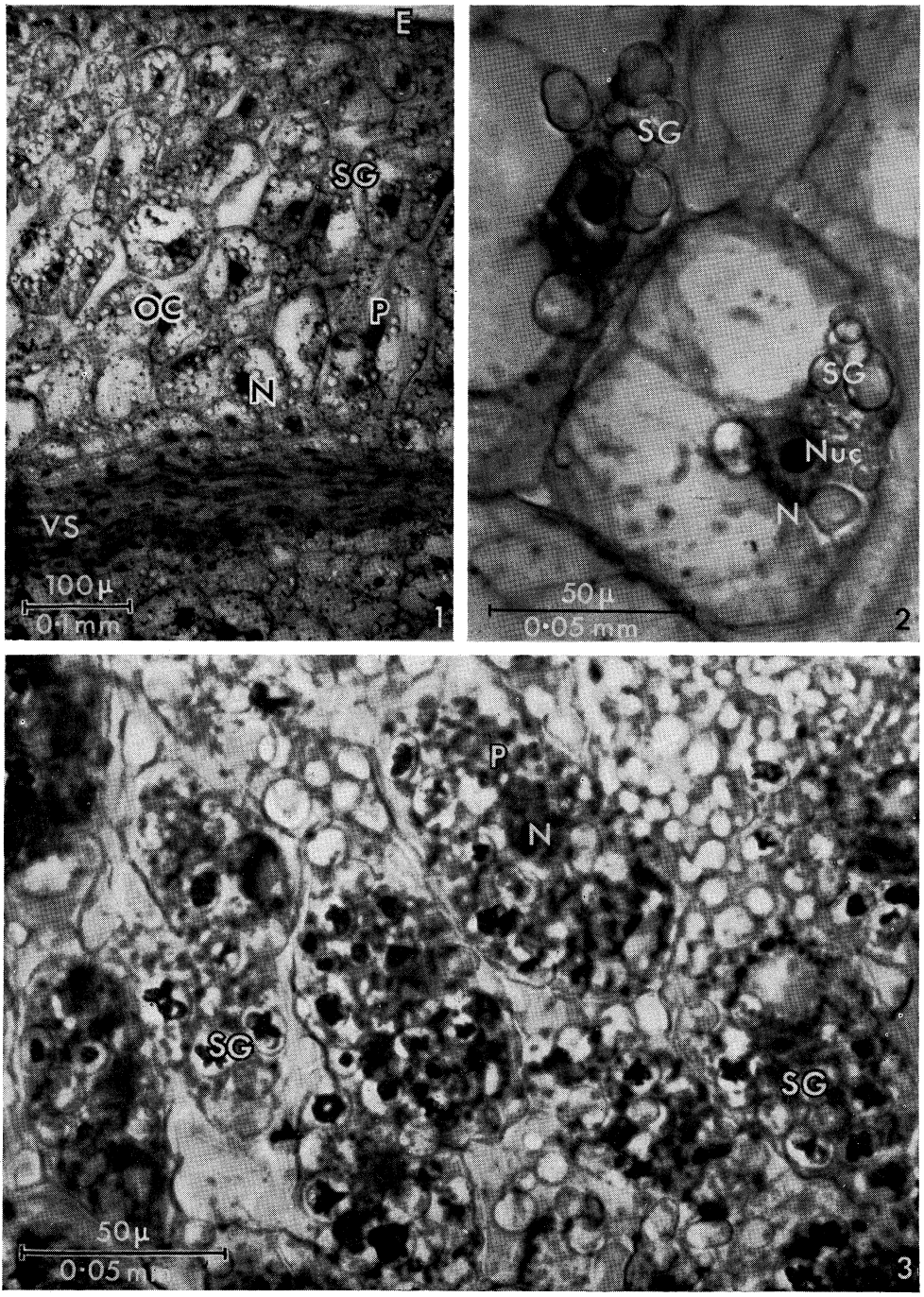


PEA COTYLEDONS DURING SEED DEVELOPMENT

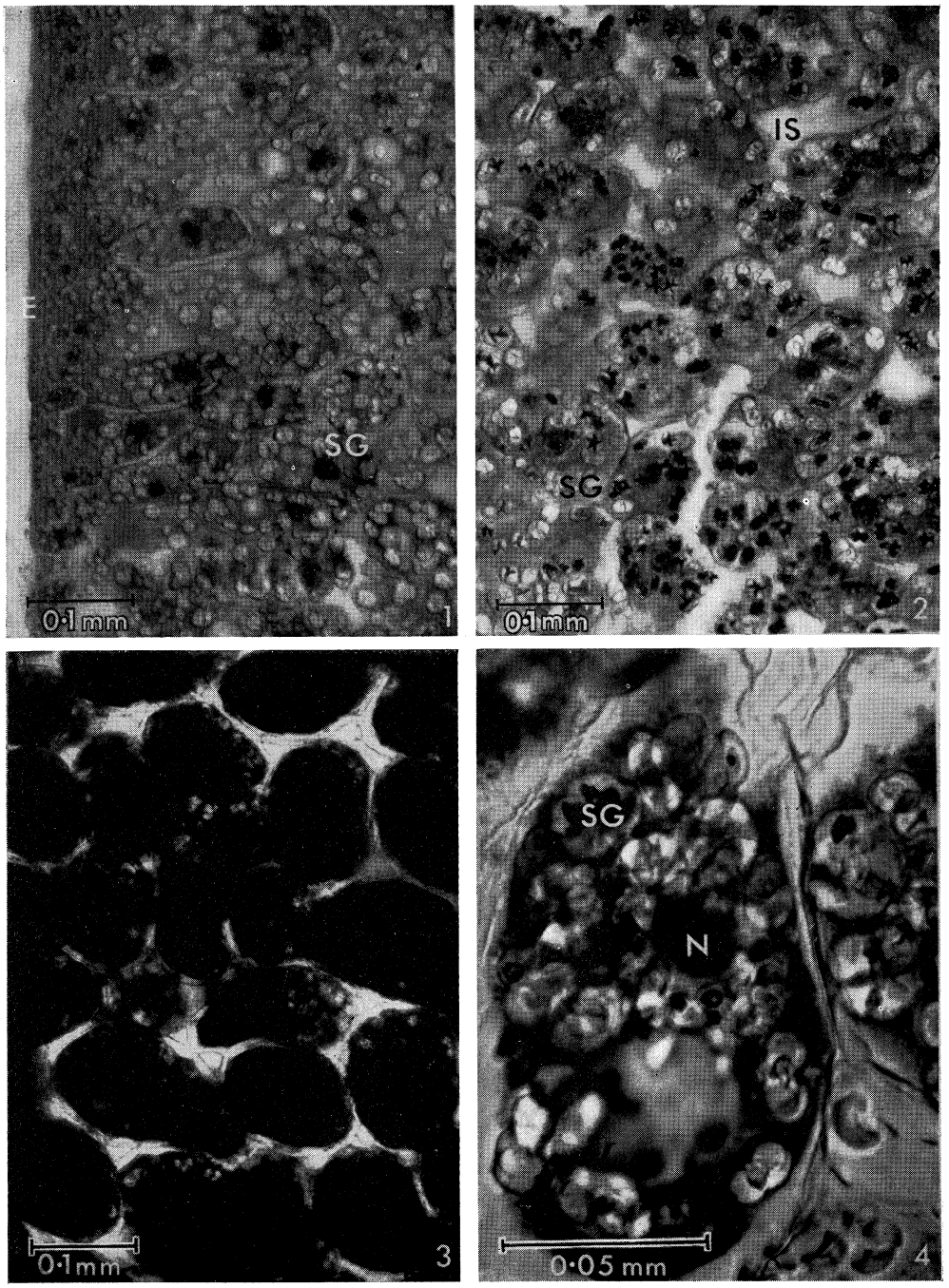


Aust. J. Biol. Sci., 1966, **19**, 49–67

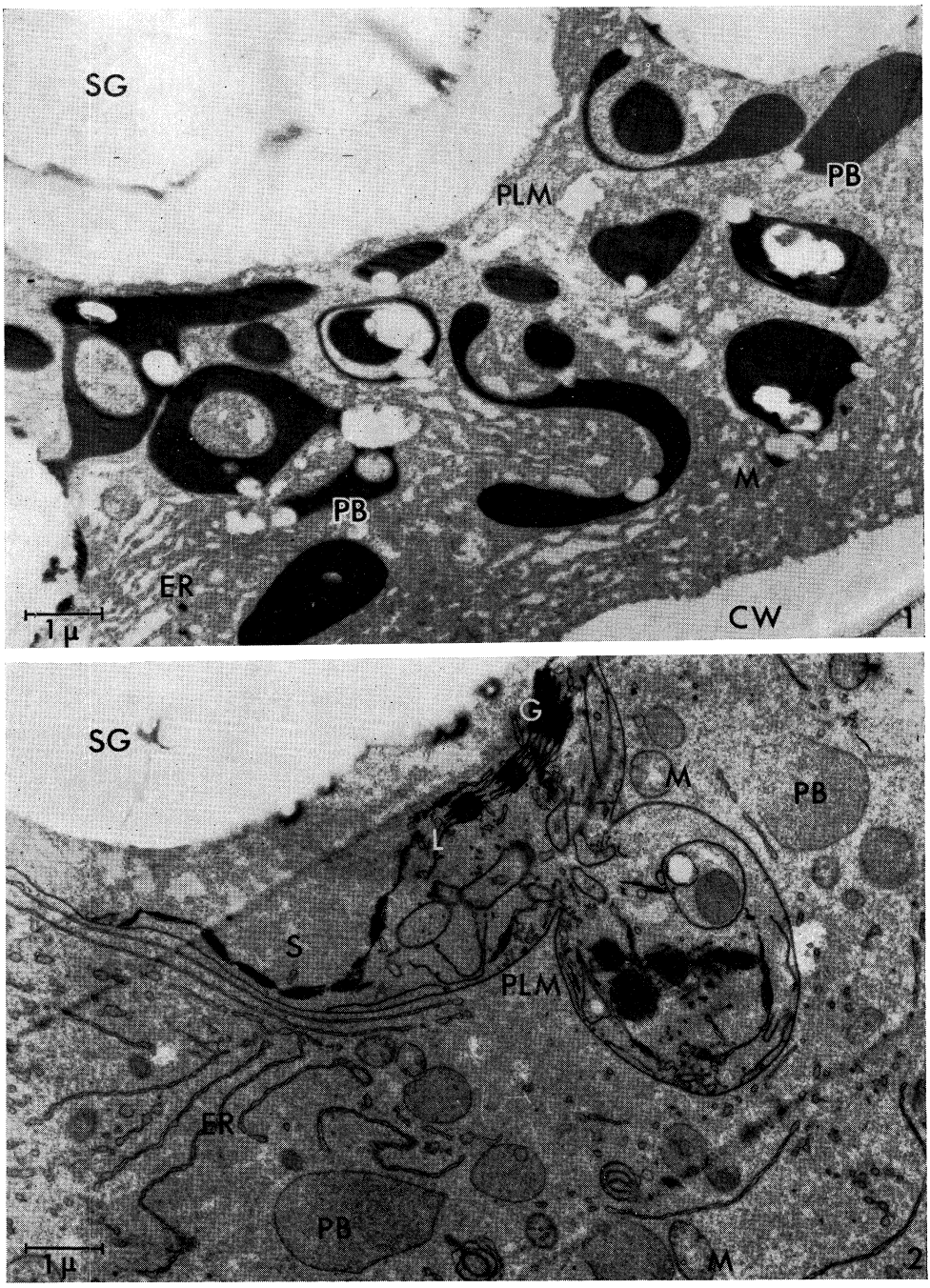
PEA COTYLEDONS DURING SEED DEVELOPMENT



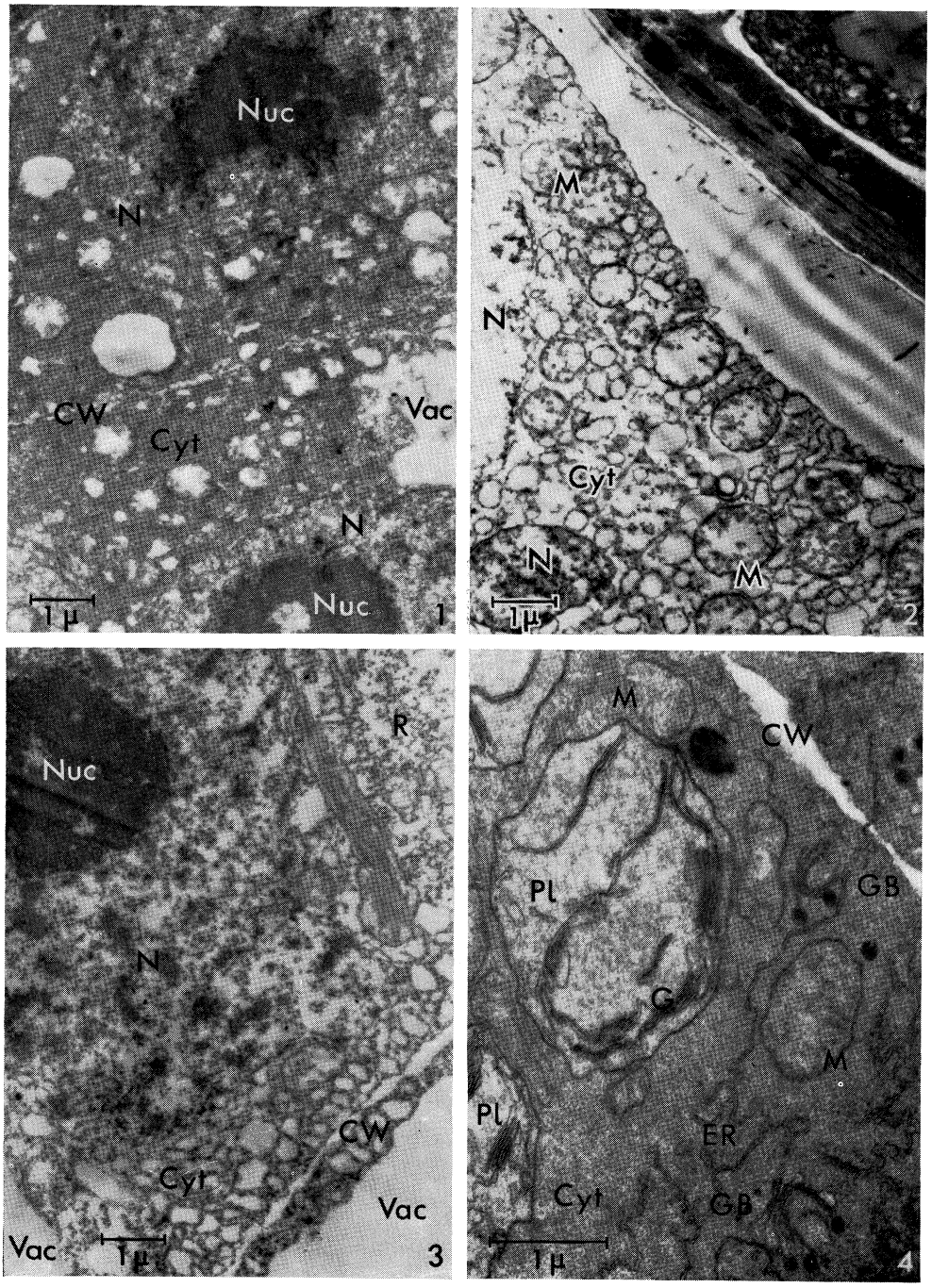
PEA COTYLEDONS DURING SEED DEVELOPMENT



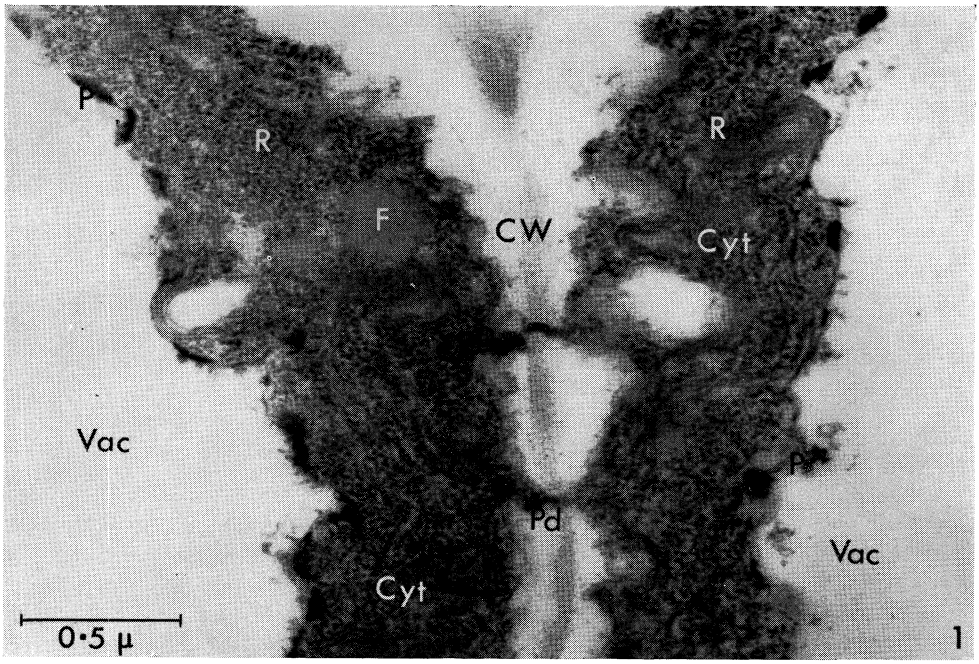
PEA COTYLEDONS DURING SEED DEVELOPMENT

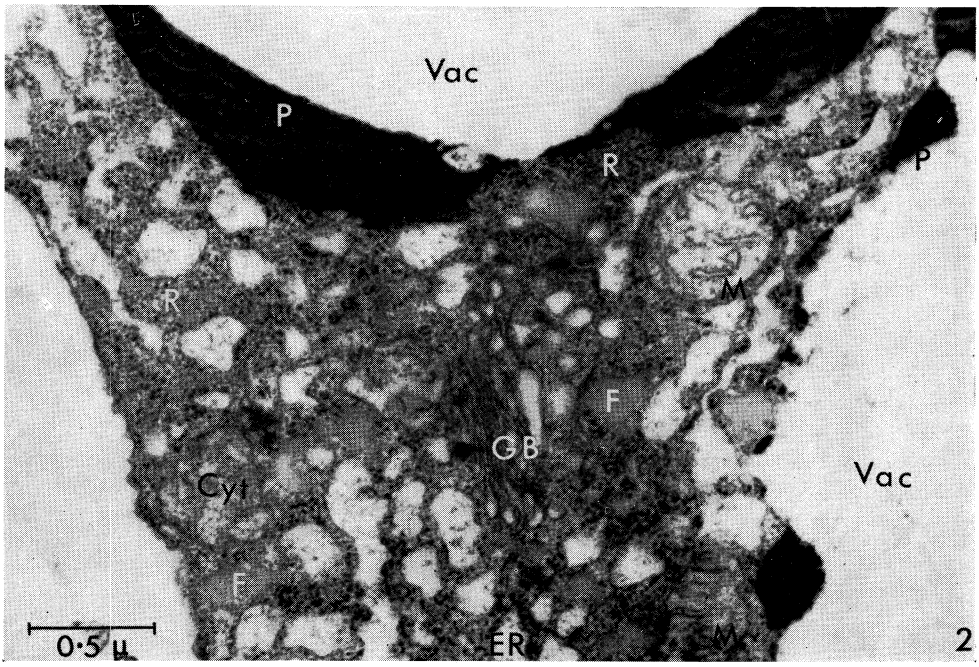


PEA COTYLEDONS DURING SEED DEVELOPMENT

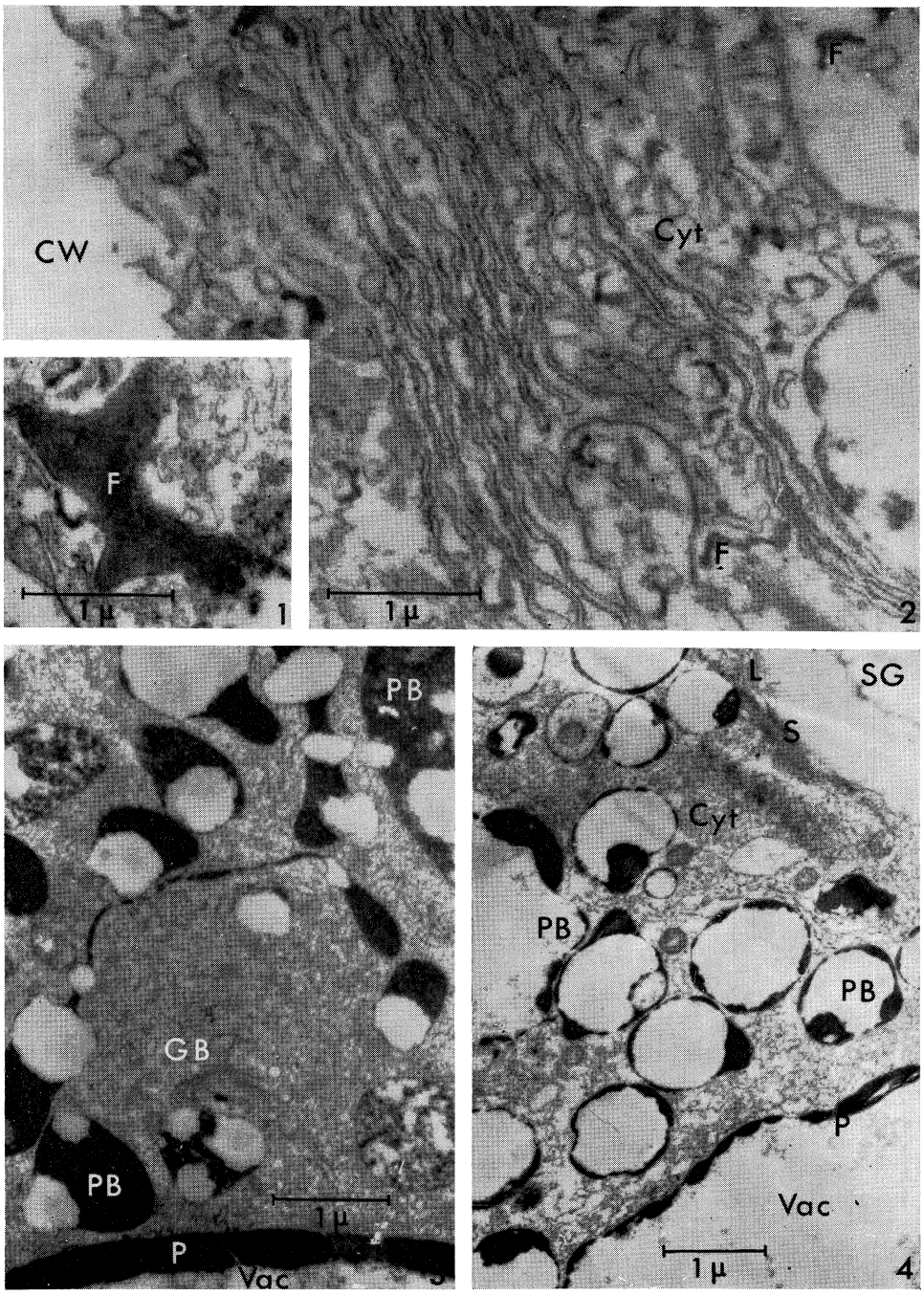


PEA COTYLEDONS DURING SEED DEVELOPMENT

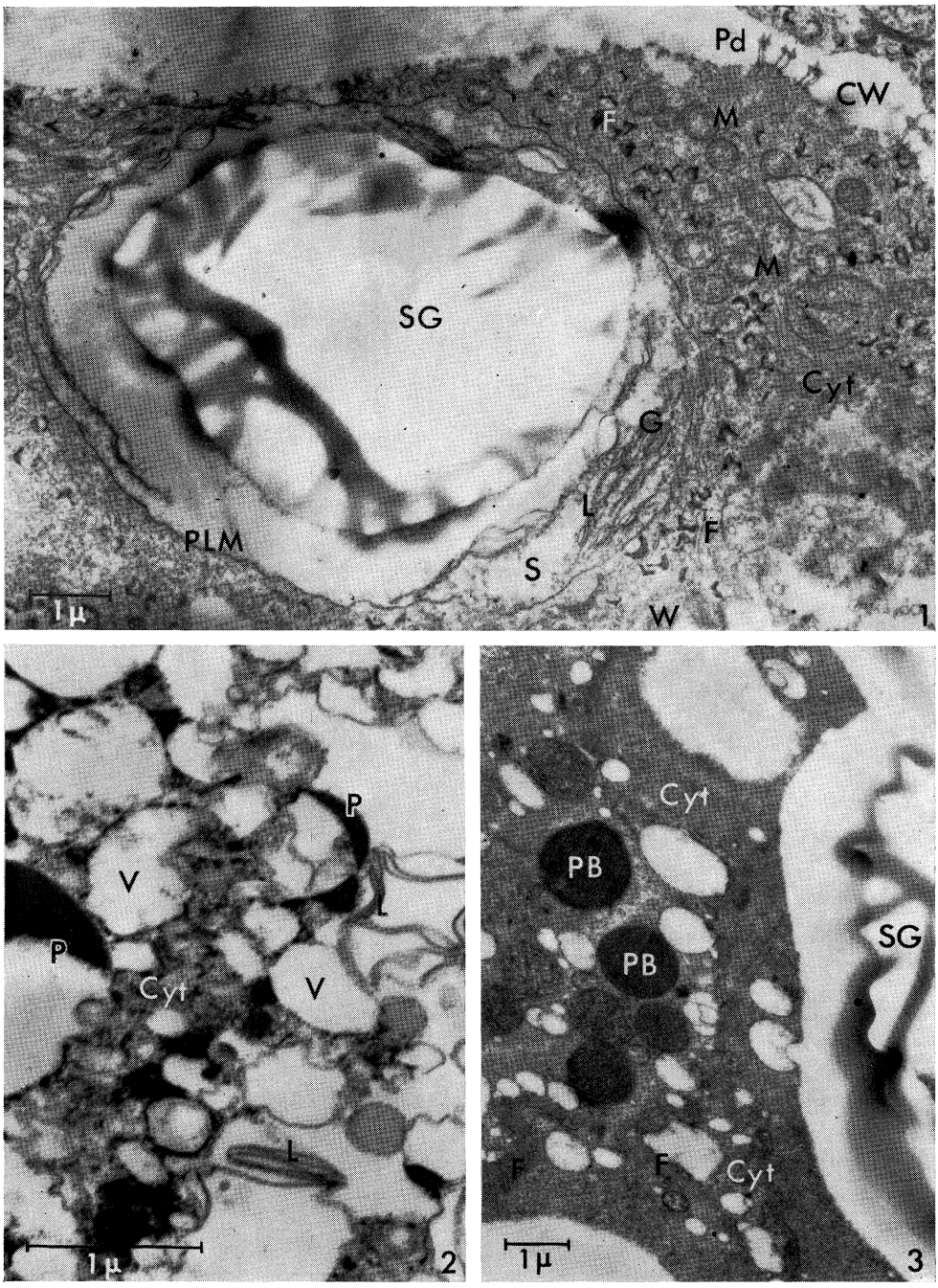




PEA COTYLEDONS DURING SEED DEVELOPMENT



PEA COTYLEDONS DURING SEED DEVELOPMENT



PEA COTYLEDONS DURING SEED DEVELOPMENT

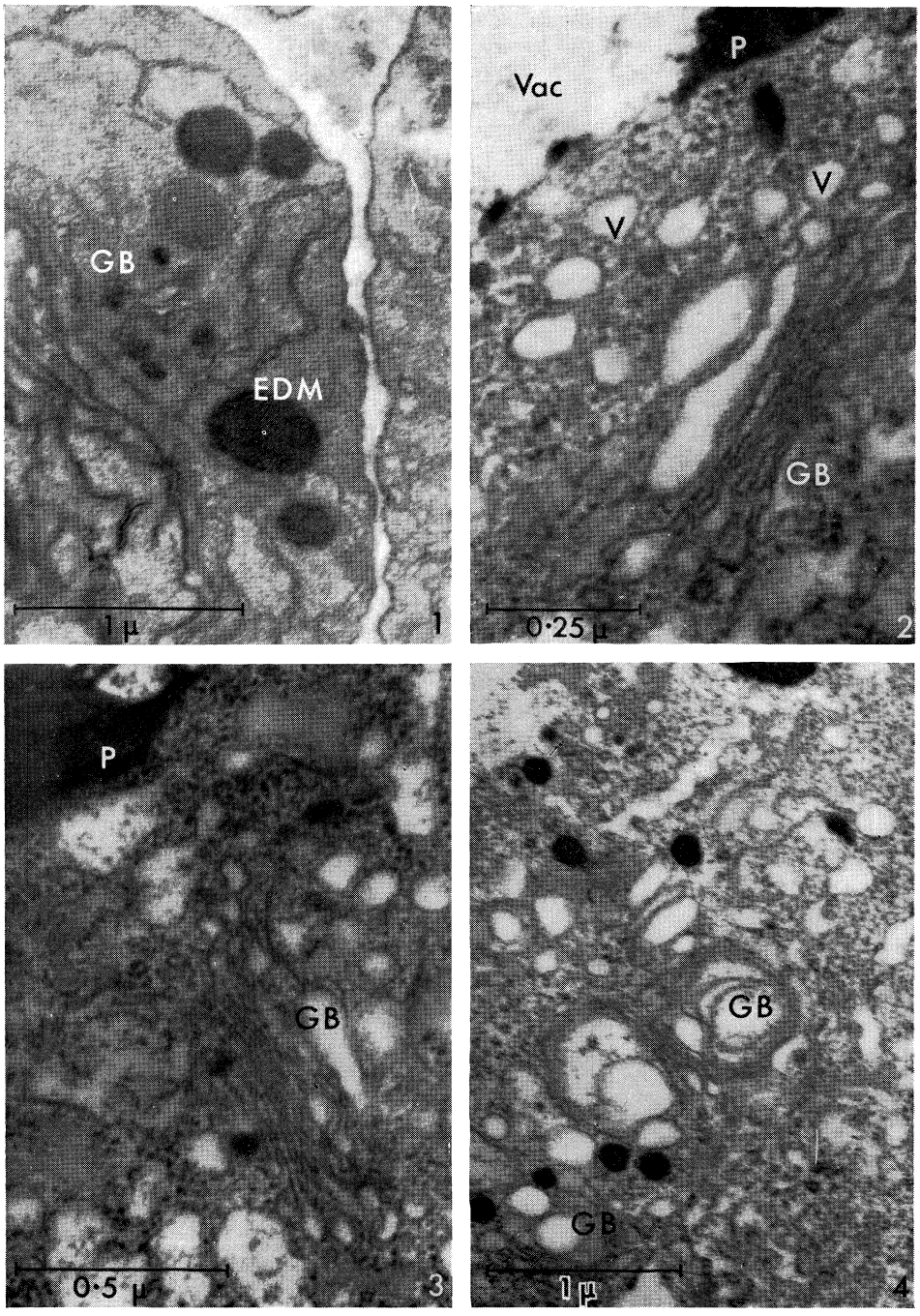


PLATE 9

Fig. 1.—After 28 days' development (end of phase 3), starch grains occupy the greater part of the volume of the plastids. A starch grain (*SG*) is separated from the limiting membrane of the plastid (*PLM*) by the stroma (*S*). The growth of the grain apparently compressed the stroma causing disruption of the lamellar system. Disorganized lamellae (*L*) and grana (*G*) are embedded in the stroma. Mitochondria (*M*) and Y-shaped fat deposits (*F*) are numerous in the cytoplasmic matrix (*Cyt*) and plasmodesmata (*Pd*) are found through the cell wall (*CW*). ×10,000.

Fig. 2.—The protoplasts of single isolated cells frequently became very disorganized during phase 3. Large vesicles (*V*) are shown to have formed in the cytoplasm (*Cyt*), former plastids are recognizable by their fragmented lamellae (*L*), and electron-dense material, presumably protein (*P*), is scattered in the cell contents. Such cells may be involved, in some way, with the transport of materials within the cotyledon tissue. ×22,500.

Fig. 3.—By the 45th day, the cytoplasm (*Cyt*) has lost most of its submicroscopic organization and the protein reserves (*PB*) have become rounded. The membranes of the endoplasmic reticulum, Golgi bodies, and the plastids are no longer apparent, and the starch grains (*SG*) are separated from the cytoplasm by "empty" spaces. Fat deposits (*F*) appear to have increased in amount, possibly from the breakdown of the lipoprotein membranes. This loss of structure is correlated with increasing dehydration of the tissue. ×7500.

PLATE 10

This series of electron micrographs is to show the secretory function, associated with the Golgi body during development of the cotyledons. The secretory function which appeared most active during phases 1 and 2 could not be correlated, however, with the large quantities of storage protein synthesized by these cells after this time

Fig. 1.—Small masses of electron-dense material (*EDM*) are associated with the Golgi body (*GB*) and are frequent in the cytoplasm of the cells of a 10-day-old embryo (end of phase 1). Fixation with potassium permanganate. ×30,000.

Fig. 2.—Protein material (*P*) is accumulating in the cell vacuoles (*Vac*) at the 20th day (end of phase 2) but no indication of an electron-dense material is seen in the vesicles (*V*) associated with the Golgi body (*GB*). ×80,000.

Fig. 3.—This electron micrograph taken at the 24th day (middle of phase 3) shows no direct communication between the vesicles of the Golgi body (*GB*) and the protein material (*P*) which was being accumulated very actively during phase 3. The vesicles of the Golgi body and the vesicles which may have been formed from the body do not contain any electron-dense material. ×52,500.

Fig. 4.—Golgi bodies (*GB*) are becoming disorganized by the 28th day with beginning of dehydration. Only a few small globular deposits of electron-dense material are still associated with them, although synthesis of storage protein was still active in the cotyledons after this time. ×25,000.