

ULTRASTRUCTURAL CHANGES IN CELLS OF PEA EMBRYO RADICLES DURING GERMINATION

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ABSTRACT

Radicles (1 mm long) of pea embryo axes, excised from dormant seeds and from seeds soaked for 80 min, 8, 12, and 48 hr, were fixed in glutaraldehyde and postfixed in OsO_4 . The nucleus was only slightly changed by 48 hr soaking, but cytoplasmic organelles underwent developmental changes. There was proliferation of endoplasmic reticulum, the appearance of dictyosomes, and an inward migration of lipid bodies. Most of these changes were observed within 8 hr after soaking started. Plastids, mitochondria, protein bodies, and protein crystalline bodies were also identified and their developmental changes were followed.

INTRODUCTION

Seed germination represents the termination of dormancy and the resumption of active embryo growth (1). At the cellular level, the process involves the development of preexisting organelles; at the molecular level, it involves primarily the synthesis (25) or activation (10, 11, 25) of messenger RNA.

Several authors have studied the fine structure of seeds of different species (18, 24, 40), but they have been mainly concerned with storage organs, e.g., the scutellum (30, 31) and aleurone cells (23) of barley, cotyledons of peas (3-5), beans (32), and cotton (44), and the endosperm of wheat (8), as well as the ultrastructural changes these organs undergo during germination. Setterfield et al. (38) studied the ultrastructure of pea and wheat embryos moistened on damp filter paper for 4 hr, but they did not follow developmental changes during germination.

The work described here was undertaken as part of an extended electron microscopic and radioautographic study of cellular changes during germination of pea seeds. The present investiga-

tion was limited to the study of the radicles ranging from 80 min to 48 hr old, because many workers (3-5, 8, 18, 23, 30-32) already have studied the ultrastructure of storage organs of germinating seeds more than 48 hr old, whereas very few studies (24, 40) have been made of the ultrastructural changes in the embryo proper, especially in the early stage germination.

MATERIALS AND METHODS

Pea seeds (*Pisum sativum* L., Century variety) were soaked in tap water at room temperature. Radicles (about 1 mm long) of embryo axes were excised from dormant seeds and from seeds soaked for 80 min, 8, 12, 17, and 48 hr in light and without aeration. Embryos that had been soaked for 80 min are referred to as 80-min embryos and, for convenience, embryos soaked for longer periods are similarly designated. Excised radicles were fixed for several hr at 4°C in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 6.8) containing a final concentration of 0.25 M sucrose. At the end of fixation, the tips were thoroughly washed with the same buffer (0.1 M at pH 6.8), postfixed for 1-2 hr in 2% unbuffered

aqueous osmium tetroxide at 4°C, dehydrated in acetone, and embedded in Epon resin as described elsewhere (46). Thin sections were cut with glass knives on the LKB Ultratome 1 (LKB Instruments, Inc., Rockville, Md.) and were stained with 20% uranyl acetate in absolute methanol (46) and lead citrate as described by Reynolds (37).

Pronase was used to extract proteins from thin sections by the method of Anderson and André (2). Thin sections were oxidized in 15% hydrogen peroxide at 40°C for 10 min and, after several washes in deionized water, incubated at 40°C for 60 min in 0.5% Pronase solution (Calbiochem, Los Angeles, Calif.) adjusted to pH 7.4. Enzyme-treated sections were washed and stained with uranyl acetate and lead citrate as described above. Electron micrographs were taken with a Philips EM 200 at 60 kv.

For light microscopic examination and histochemical localization of proteins, sections were cut at 2 μ from the same Epon-embedded radicles, stained with mercuric bromophenol blue (26) and periodate-Schiff's reagent (21), and mounted in Zeiss phase-mounting medium L15 (Carl Zeiss Canada Ltd.).

Since 1 mm long pea radicles include a variety of tissues, cells in the cortex near the apical meristem (arrow in Fig. 1) only were examined to follow developmental changes occurring during soaking.

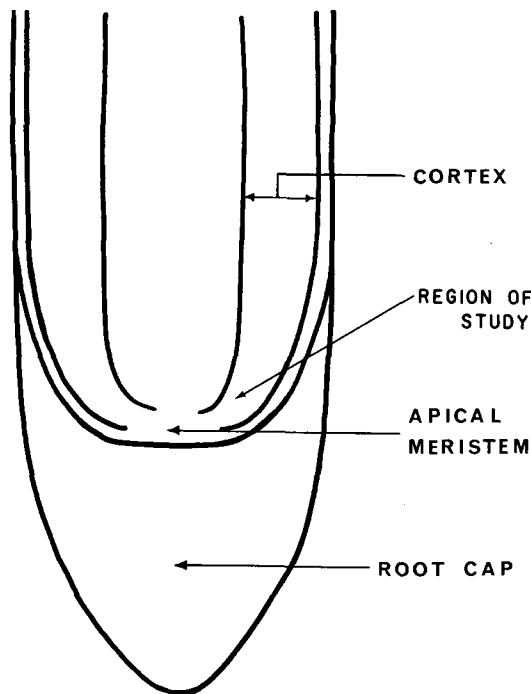


FIGURE 1 Diagram of a pea root tip. The arrow indicates the region where cells were studied.

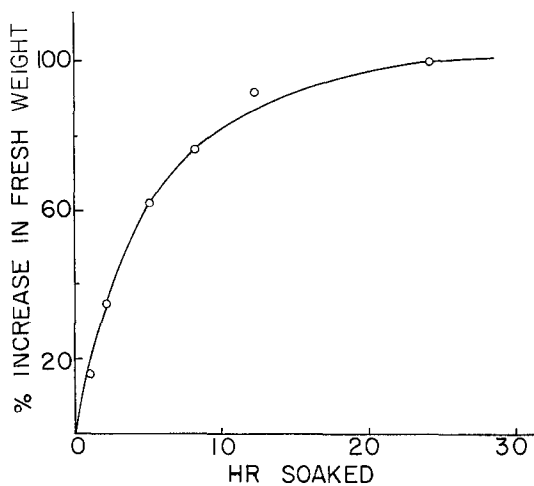


FIGURE 2 The rate of imbibition by dormant pea seeds.

OBSERVATIONS

Upon soaking, pea seeds imbibed water and increased in fresh weight, as shown in Fig. 2. The rate of water uptake was almost linear for the first 5 hr of soaking, but gradually slowed down thereafter. After soaking for 12 and 24 hr, the fresh weight was increased by 92 and 100%, respectively, and was changed little for the next 24 hr of soaking.

Although the cells of both dormant and soaked radicles contained the nucleus, mitochondria, plastids, rough and smooth endoplasmic reticulum (ER), ribosomes, protein bodies, and other cellular inclusions, obvious developmental changes of cytoplasmic organelles did occur during soaking.

Nucleus

The nuclei of dormant and soaked radicles were irregular in outline (Fig. 4). The nucleus was surrounded by a double membrane that contained nuclear pores. Because of the irregular shape of the nuclei, thin sections showing a tangential view of the pores were frequently observed.

Plastids

The presence of phytoferritin made it easy to identify the plastids and to follow their developmental changes during germination (Figs. 4, 5). The plastids of radicles of dormant embryos often contained starch grains and osmiophilic granules, but were almost free of lamellae and peripheral vesicles (Fig. 5). Osmiophilic granules in the

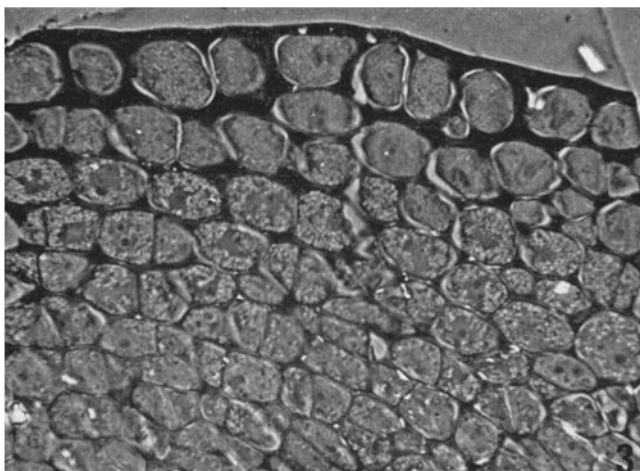


FIGURE 3 Cross-section (somewhat oblique) of a radicle soaked for 80 min. A cut was made just above the apical meristem, showing epidermis and cortex. Cell walls were stained for carbohydrate, but protein bodies were not stained. Periodate-Schiff's reaction. $\times 100$.

plastids of dormant radicles were usually gathered in arrays in the region where phytoferritin was absent. Development of lamellae and peripheral vesicles were observed in the plastids of the radicles of 8-hr embryos, but thereafter the plastids were little changed. Smooth ER was often seen to surround the plastids in dormant radicles (Figs. 4, 5), but this was not the case in the radicles of 48-hr embryos.

Mitochondria

Numerous mitochondria were found in the cytoplasm of both dormant (Figs. 4, 6) and soaked radicles (Fig. 7), and showed the following ultrastructural features typical of mitochondria (Figs. 6, 7): a surrounding double membrane, cristae, matrix, and occasional small dense granules. When mitochondria of dormant embryos were compared with mitochondria of soaked embryos, however, the following differences in their ultrastructure were observed: (a) cristae were, in general, better defined in mitochondria of soaked seeds (Fig. 7) than in those of dormant seeds (Fig. 6), probably as a result of the clustering of electron-opaque material around the cristae of the mitochondria of dormant seeds; although the number of cristae appeared unchanged; (b) the matrix of mitochondria appeared to contain less electron-opaque material in soaked seeds (Fig. 6) than in dormant seeds (Fig. 7); and (c) mitochondria were rounded in dormant embryos but appeared elongated in soaked embryos.

Dictyosomes

Dictyosomes were found in the cytoplasm of embryos soaked for longer than 8 hr (Fig. 9), and

the number of dictyosomes per cell appeared to increase as germination proceeded. Although no dictyosomes as such (cisternae, Golgi vesicles) were found in the cytoplasm of dormant and 80-min embryo radicles, stacks of flattened sacs occurred in the vicinity of the cell wall (Figs. 8, 11). If these stacks are regarded as prodictyosomes (28), the development of dictyosomes from prodictyosomes would constitute a developmental change during germination.

Endoplasmic Reticulum

Both smooth and rough ER occurred in the cytoplasm of the radicles of dormant embryos, but they were sparse. Although the two types of ER usually occurred independently, a continuum between the two types of ER was also observed in dormant embryos (Fig. 10). While rough ER always occurred as short segments (Fig. 10), smooth ER was either short (Fig. 11) or long, and occasionally branched (Figs. 4, 5). The latter branched portions were seen to surround some plastids (Figs. 4, 5). One of the most obvious developmental changes taking place in the cytoplasm during soaking was an extensive and rapid proliferation of ER. Proliferation, already observed in the cytoplasm of 8-hr embryos, progressed as soaking continued (Fig. 12).

Ribosomes

Ribosomes were present in the cytoplasm of both dormant (Figs. 4-6, 10, 15) and soaked radicles (Figs. 7, 9, 14, 16), but the number per unit volume decreased as the length of soaking increased.

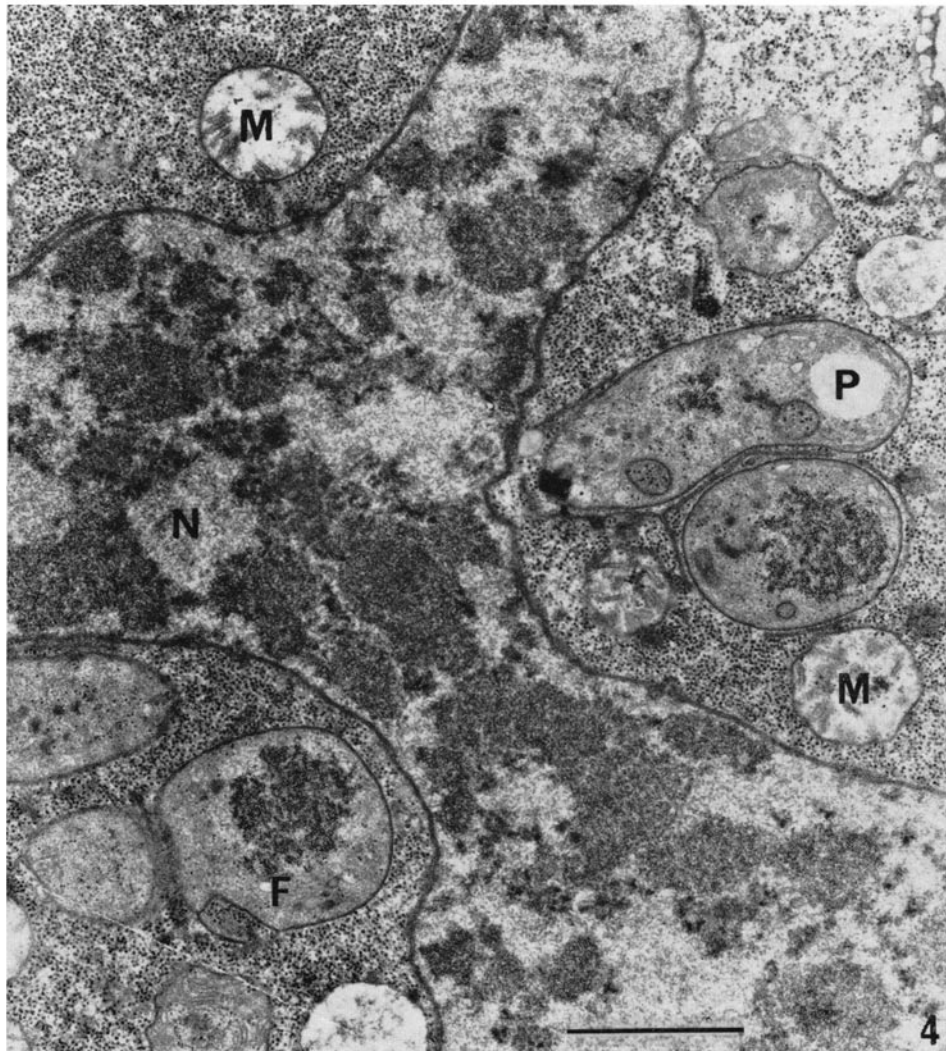


FIGURE 4 Electron micrograph of a cell of a dormant embryo radicle. Note irregular shape of the nucleus and the absence of ER from a large part of the cytoplasm. The following labels are used for Figs. 4-24. *D*, dictyosomes; *K*, protein crystal; *M*, mitochondria; *Mv*, multivesicular bodies; *P*, plastids; *W*, cell wall; *F*, phytoferritin; *L*, lipid bodies; *Mb*, microbody-like organelles; *N*, nucleus; *V*, protein bodies; *Y*, yarnball-like structure. Scale line = 1 μ . \times 22,600.

Lipid Bodies

Lipid bodies in dormant embryos appeared circular and smooth and were bounded by a single membrane (Figs. 8, 13, 15). These lipid bodies were found closely packed against each other and were confined to regions near the cell surface (Fig. 15) or near the plastids in radicles of dormant, 80-min, 8-, and 12-hr embryos. In the

radicles of 48-hr embryos they were no longer confined to regions near the cell surface (Figs. 12, 14-16, 19). As shown in Fig. 13, short strands of smooth ER appeared to radiate from lipid bodies and run from one body to another. As germination progressed, the ER became elongated but appeared to remain attached to lipid bodies, even after these lipid bodies migrated inward through the cytoplasm (Fig. 14).

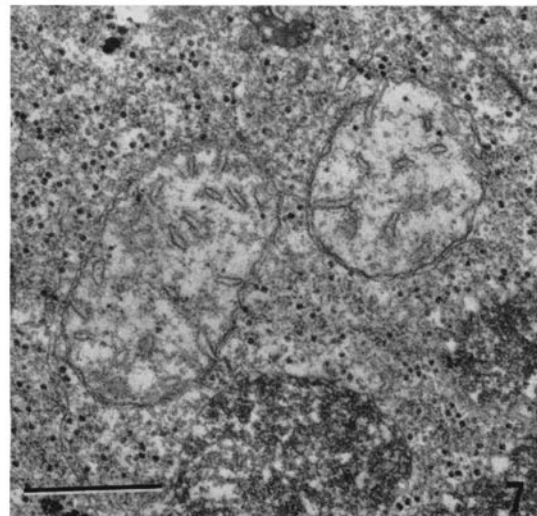
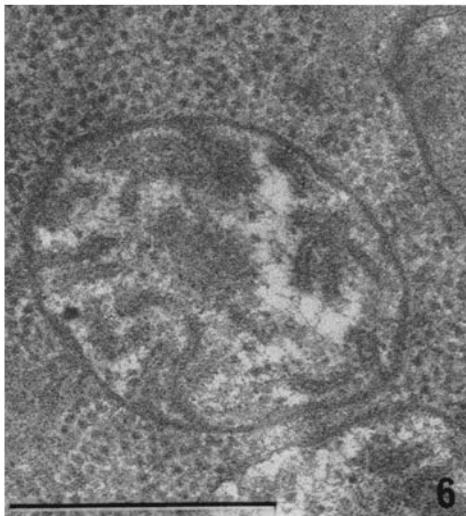
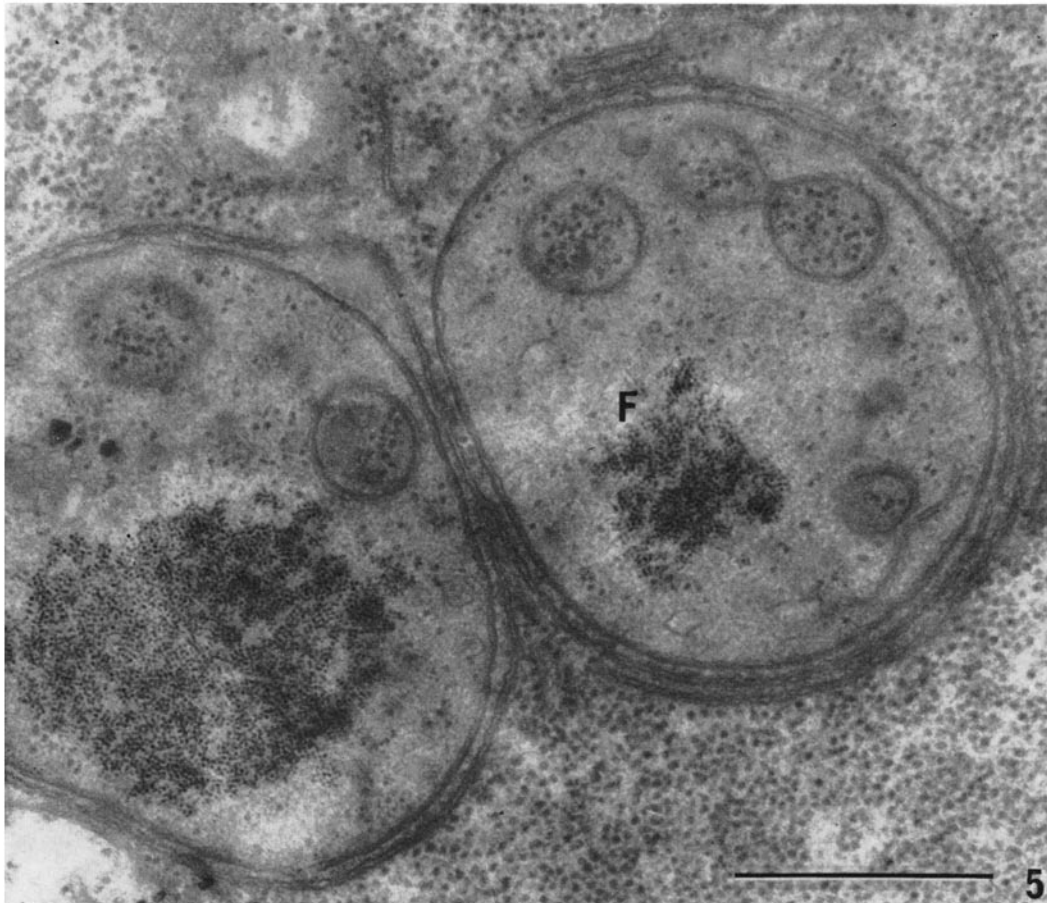


FIGURE 5 Plastids of a dormant embryo radicle. Smooth ER is branching and surrounds the plastids. Scale line = 0.5μ . $\times 68,000$.

FIGURE 6 A mitochondrion of a dormant embryo. Scale line = 0.5μ . $\times 71,000$.

FIGURE 7 Mitochondria of a 17 hr embryo. Scale line = 0.5μ . $\times 36,000$.

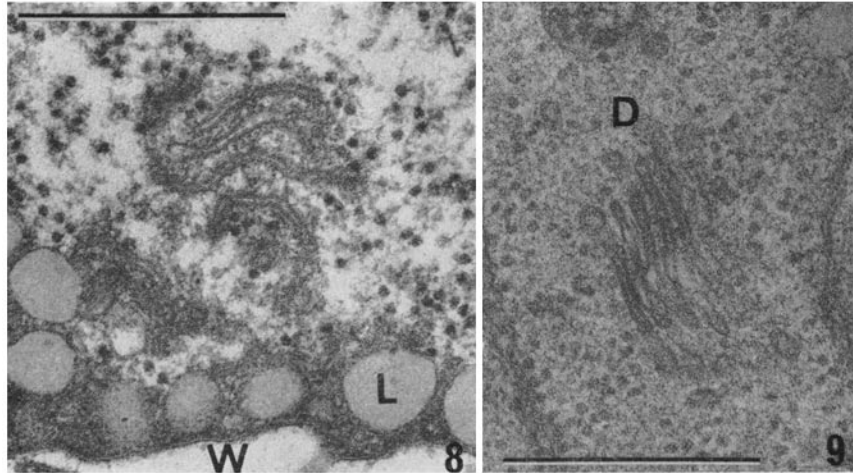


FIGURE 8 Stacks of flattened sacs in the cytoplasm of a dormant embryo. Scale line = 0.5μ . $\times 72,500$.

FIGURE 9 Dictyosome in the cytoplasm of an 8 hr embryo radicle. Scale line = 0.5μ . $\times 67,500$.

Protein Bodies

Numerous vesicles were present in the cytoplasm of dormant embryos. Most of the vesicles stained with mercuric bromophenol blue, a protein stain, but not with the Schiff's reagent (Fig. 3) and were, accordingly, identified as protein bodies. These protein bodies were surrounded by a unit membrane and contained a meshwork of electron-opaque materials. The amount of these materials varied widely. Some protein bodies were uniformly filled with material while others were empty except for a few threads, but most bodies contained intermediate amounts. A few contained a structure resembling a ball of yarn; this structure remained unchanged during 48 hr of soaking (Figs. 17, 18). As soaking progressed, the electron-opaque protein in these bodies disappeared, leaving progressively more electron-lucent regions (Figs. 17, 19). The contents of these protein bodies are assumed to comprise reserve proteins, for, while it was possible to stain them with mercuric bromophenol blue, the electron-opaque material was not extracted by Pronase.

Protein Crystals

Crystalline bundles with needle-like structures were found in the cytoplasm of dormant as well as soaked embryo radicles (Figs. 22, 24), but they were found more frequently in dormant than in soaked radicles. The length of the structures varied widely, but the longer the period of soaking, the

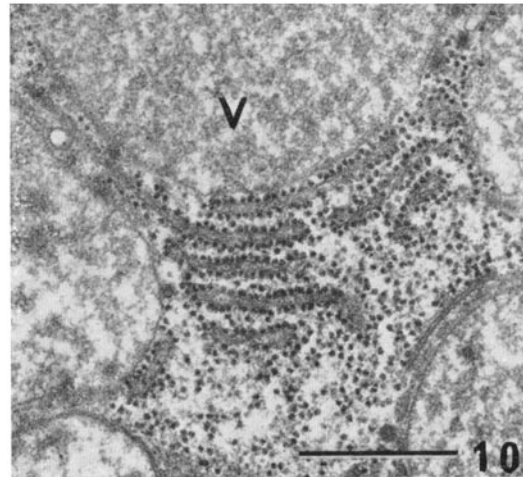


FIGURE 10 Short segments of rough ER in the cytoplasm of a dormant embryo. Note that one end of the rough ER is free of ribosomes. Scale line = 0.5μ . $\times 42,000$.

shorter was their length. The distribution of these structures was not limited to the cytoplasm, as they were found occasionally in the nucleus; since Pronase extracted the structures from thin sections (Fig. 23), it is concluded that they are protein.

Microbody-like Organelles and Multivesicular Bodies

Microbody-like organelles were frequently observed in embryos soaked for more than 8 hr

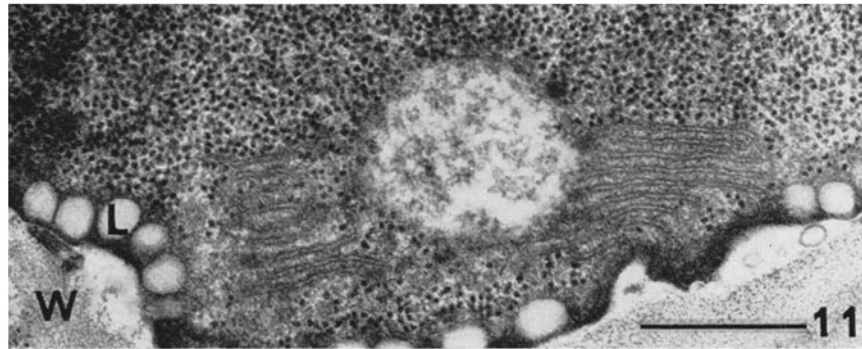


FIGURE 11 Short segments of smooth ER running parallel in the vicinity of the cell wall in a dormant radicle. Scale line = 0.5μ . $\times 44,000$.

(Figs. 14, 21). The organelles were finely granular, moderately electron opaque, bounded by a single membrane, and closely associated with ER. Multivesicular bodies were also found in the vicinity of microbody-like organelles in 8-hr embryos, but they were very rare (Fig. 21).

DISCUSSION

The increase in fresh weight of pea seeds is related to the duration of soaking, which is used in turn as a reference for changes in the ultrastructure during soaking. When the fresh weight was doubled, after 24 hr of soaking, no further increase in the fresh weight was observed for the next 24 hr. This is interpreted with the premise that all the imbibing molecules present in dormant pea seeds were completely hydrated during the first 24 hr and that a further increase in fresh weight would only be possible when newly imbibing molecules are synthesized. The synthesis of newly imbibing molecules apparently did not occur during soaking. In this connection it should be noted that, although 3–5 hr of soaking are required for pea seed germination, a prolonged soaking inhibits germination (33).

Pea radicles increased in volume during soaking, but such an increase is harder to determine than an increase in fresh weight. The increase in volume during soaking would mainly be due to hydration of cells rather than to enzyme- or metabolism-mediated cell elongation and cell division. This view seems to be in agreement with radioautographic observations (47), that no tritiated thymidine was incorporated into the nuclei of cells, during 48 hr of soaking, and that emergence of radicles from the seed coat, the morphological defi-

nition of germination, was not observed at the end of 48 hr soaking.

Two problems were realized during the course of the present study. The first was the uneven degree of hydration across the radicles during the early stages of soaking. Since imbibition is a slow process, it is probable that the cells of the inner cortex, for example, remained dehydrated, while the epidermal cells were fully hydrated. But, because of an uneven degree of hydration during early stages of soaking, cells in one region could be metabolically active, while those in another region could be inactive. Thus, radicles in the early stages of soaking do not represent uniform systems. For this reason, and because of the presence of a variety of tissues in the 1 mm long radicles, it was desirable to use cells from the same region in each case.

The second problem was whether aqueous fixatives could be used to preserve the "true" structure of dehydrated cells. The moisture content of the pea seeds used ranged from 6 to 10%. Upon contact with aqueous fixatives, cellular organelles could absorb water, swell, and consequently, might look different from the same structures as they exist in the cells of dormant pea seeds. Perner (36), for this reason, fixed dried dormant pea radicles with the vapor of osmium tetroxide for several months. The ultrastructure of cellular organelles fixed in this way (36) differed in the following manner from that of organelles fixed in aqueous osmium tetroxide: (a) cellular organelles, in general, exhibited low contrast; (b) membranous structures were shrunken and had a negative image; (c) ribosomes were not seen in the cytoplasm, and the mitochondrial cristae were un-

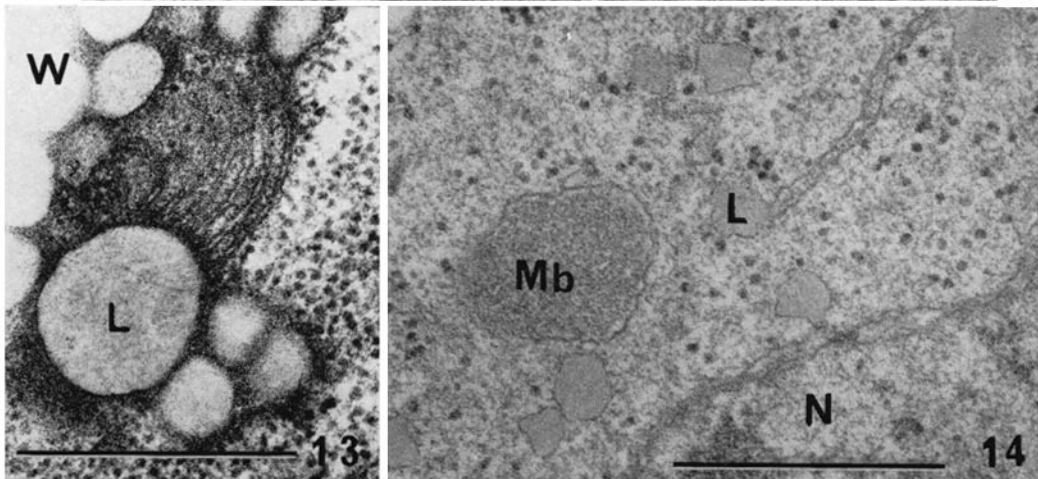
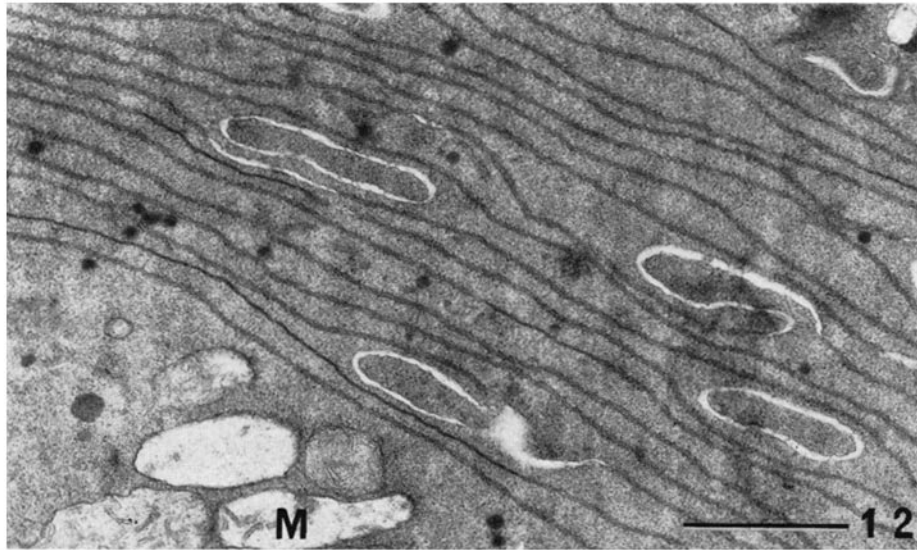


FIGURE 12 Electron micrograph of a 48 hr embryo radicle showing inwardly migrated lipid bodies (dark spots) and strands of ER running more or less in parallel. Scale line = 1μ . \times 21,800.

FIGURE 13 Electron micrograph of an 8 hr embryo showing lipid bodies, from one of which six strands of smooth ER appear to emerge. Scale line = 0.5μ . \times 73,500.

FIGURE 14 Electron micrograph of a 48 hr embryo showing a microbody-like organelle and migrated lipid bodies. Scale line = 0.5μ . \times 71,000.

recognizable; and (d) the reserve proteins in protein bodies were in the crystalline state. The structure of cellular organelles, as shown by Perner (36), might be closer, if not identical, to the "true" structure of the organelles as they exist in dormant pea seeds.

With Perner's work as reference, it seems reasonable to compare the ultrastructure of cellular

organelles in dormant pea radicles with that of organelles in soaked pea radicles for the study of developmental changes in the ultrastructure.

During soaking, the nucleus showed very little change, except in shape. The plastids also showed very little change throughout the soaking period. This observation is in agreement with others (24). The appearance of peripheral vesicles and short

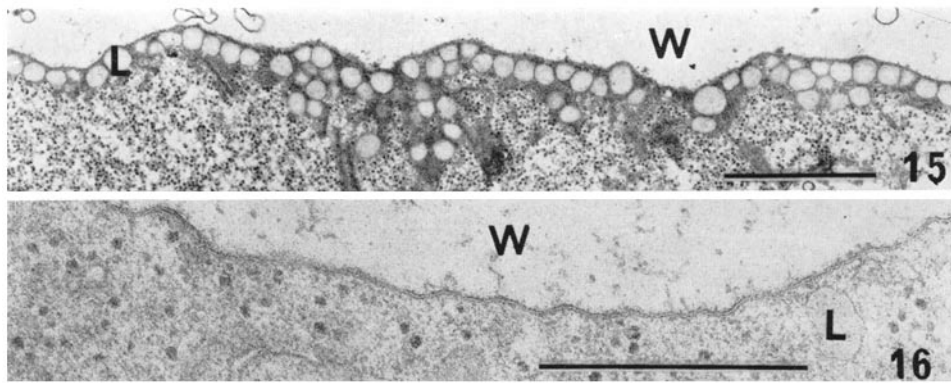


FIGURE 15 A layer of lipid bodies beneath the plasma membrane in a dormant embryo. Scale line = 1μ . $\times 20,300$.

FIGURE 16 Electron micrograph of a 48 hr embryo showing the plasma membrane. Note that all but a few lipid bodies have migrated. Scale line = 0.5μ . $\times 70,000$.

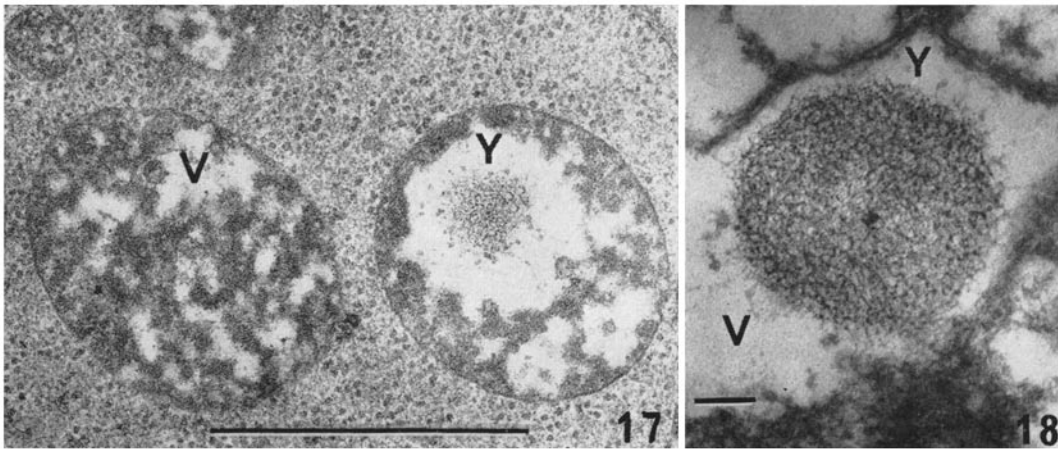


FIGURE 17 Protein bodies in a 12 hr embryo showing that protein has started to disappear. Note a yarnball-like structure in the protein body. Scale line = 1μ . $\times 42,000$.

FIGURE 18 A yarnball-like structure in a protein body. Scale line = 0.1μ . $\times 83,000$.

fragments of lamellae in the plastids marked the only structural changes observed during germination, suggesting that the plastids did not progress beyond the proplastid phase of development (15). Phytoferritin particles and osmiophilic granules also persisted with little change.

Plastids fixed with the vapor of osmium tetroxide (36) were surrounded by shrunken membranes and contained, like those fixed in aqueous osmium tetroxide, osmiophilic granules and phytoferritin.

Mitochondria in dormant radicles fixed in

aqueous osmium tetroxide are rounded (Fig. 6), or elongated, as reported by others (24, 40) who also used aqueous fixatives. However, the round shape of mitochondria seems to be an artifact of aqueous fixation as mitochondria fixed with osmium tetroxide vapor were angular and star-shaped (36). Mitochondria fixed with the vapor, however, did not reveal cristae clearly enough to allow a comparison with mitochondria fixed in aqueous fixatives, but they differed from mitochondria of the present study (Fig. 6) in having an

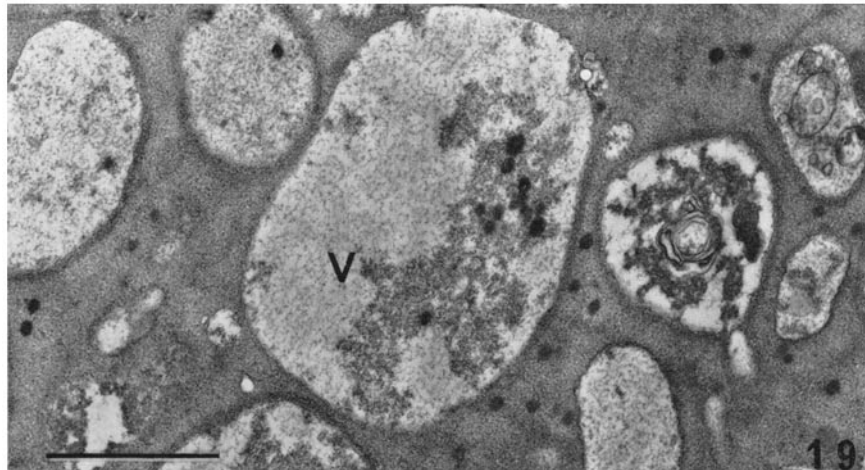


FIGURE 19 Electron micrograph of a 48 hr embryo. Note that a great part of the protein has disappeared from the protein bodies. Scale line = 1 μ . \times 23,000.

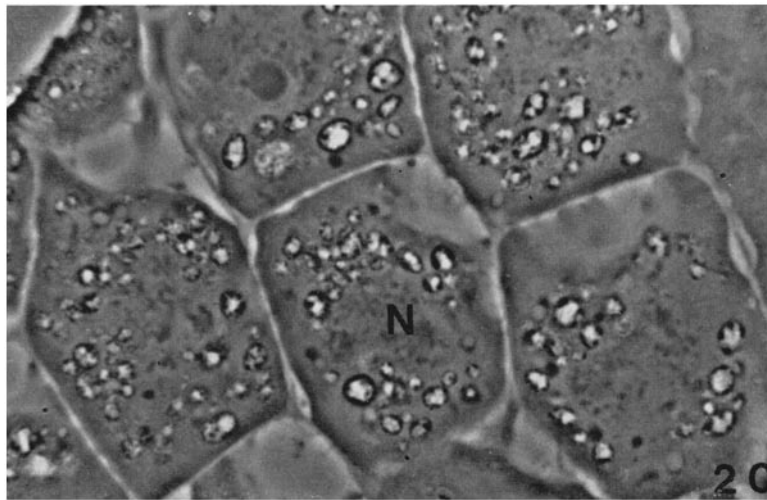


FIGURE 20 Cells in the arrow-marked region in Fig. 1 after 48 hr of soaking. Cells were enlarged and protein bodies were vacuolated and stained positively for protein. Mercuric bromophenol blue. \times 1,500.

electron-lucent area in the center. Although differences in appearance between mitochondria of dormant (Fig. 6) and of soaked radicles (Fig. 7) are listed under Observations, it is hard to speculate as to the causes of such differences.

It appears that dormant plant embryos may or may not contain dictyosomes, depending on the species and conditions under which the seeds mature. Some workers have found dictyosomes in dormant embryos (24, 32), while others have

found them only after germination has started (23, 40).

Since Bain and Mercer (3) found dictyosomes in developing pea cotyledons, as did Buttrose (8) in developing wheat endosperm, one might have expected to find dictyosomes in the cytoplasm of dormant pea embryos. My failure to find them does not necessarily prove their absence in dormant pea embryos, but implies either that dormant pea embryos contain very few dictyosomes

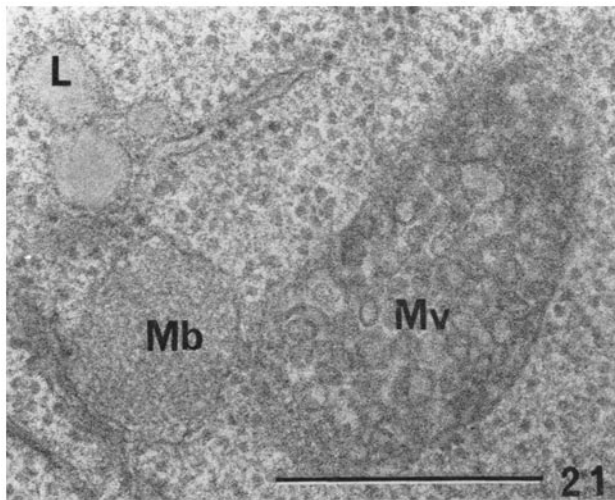


FIGURE 21 Electron micrograph of an 8 hr embryo showing a microbody-like organelle, a multivesicular body, lipid bodies, and ER. Note a close association between the microbody-like organelle, a multivesicular body, lipid bodies, and ER. Note a close association between the microbody-like organelle and the multivesicular body. Scale line = 0.5μ . $\times 70,000$.

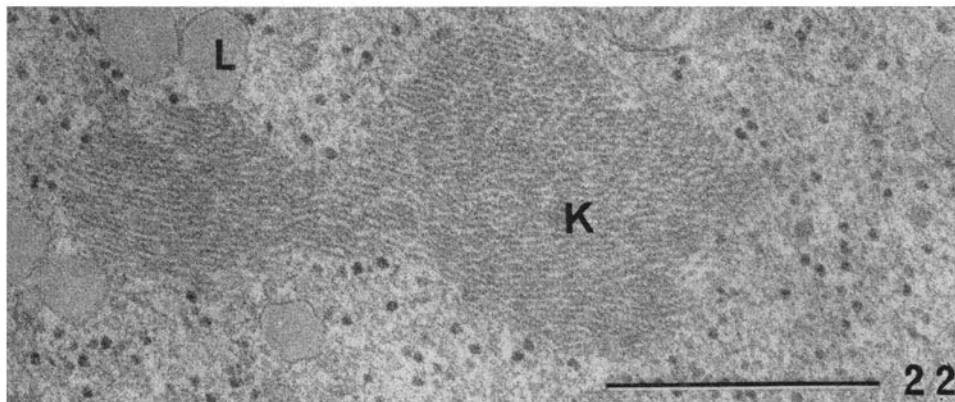


FIGURE 22 Electron micrograph of a 48 hr embryo showing protein crystal. Scale line = 0.5μ . $\times 71,000$.

or that dictyosomes exist as prodictyosomes as described in Observations (Figs. 8, 11). In either case, the development of dictyosomes and the proliferation of ER constitute obvious changes occurring in the cell during the period of soaking.

The stacks of flattened sacs in Figs. 8 and 11 can be interpreted either as stacks of short elements of smooth ER or as prodictyosomes without Golgi vesicles. It is very hard to tell when these stacks of smooth ER cease being ER and become prodictyosomes. If these stacks are regarded as prodictyosomes, dictyosomes in germinating pea seeds must be produced as a result of flow and reorientation of smooth membranes (28).

Dormant pea seeds had three kinds of preformed ER in the cytoplasm: short segments of rough ER

(Fig. 10), smooth ER surrounding the plastids (Fig. 5), and smooth ER attached to lipid bodies (Fig. 13).

Lipid bodies have been identified under different names: spherosomes (20, 31, 34), intercellular network (7), vacuole (30), lipid globules (32), and "osmium dense bodies" (24). The term "lipid body" is used in this paper (Mollenhauer [27]) to reflect the chemical components of the bodies.

Mollenhauer (27) observed that lipid bodies of bean cotyledons were converted into an extensive membranous system with an appearance of smooth ER during the initial stages of germination. Since short segments of smooth ER remained attached to a lipid body (Fig. 14) and appeared to have grown out from the membrane limiting the lipid

bodies, it is tempting to conclude that lipid bodies are involved in ER formation.

It appears that lipid bodies are organelles of lipid storage (23) and supply lipids to the membrane systems, especially to ER. Being closely packed against the cell wall, they probably prevent the loss of moisture from the cells. Inward movement of lipid bodies from the cell periphery, as shown here, was also observed in the scutellum of barley by Nieuendorp and Buys (31).

Chapman and Rieber (9) reported that the number of ribosomes per unit volume decreased as the length of soaking increased (Figs. 11 and 14). This is probably due to an enlargement of the cell without a net synthesis of ribosomes. In this study, contrary to their findings (9), ribosomes were not confined to limited cytoplasmic regions of dormant seeds but were distributed throughout the cytoplasm. However, the apparently uniform distribution of ribosomes throughout the cytoplasm may be the result of aqueous fixation.

On the basis of histological studies (Figs. 3, 20), protein bodies were identified. The presence of protein in protein bodies of *Yucca* and lettuce seeds was also demonstrated histochemically by Horner and Arnott (17) and Paulson and Srivastava (35), respectively. However, Pronase failed to extract the content of protein bodies from thin sections. The failure is probably due to alterations in pro-

tein structures in the bodies produced as a result of fixation, dehydration, and embedding.

Vacuolization of protein bodies is interpreted either as digestion and utilization of storage proteins, or as extraction of proteins during soaking and fixation, or as a combination of the two. If vacuolization results from digestion, then hydrolytic enzymes are likely to be located within the bodies, because the membrane bounding the bodies appeared to remain intact. In this sense, the bodies could be considered autolytic and to act as lysosomes (45).

As a result of the loss of storage proteins at the end of 48 hr of soaking, the protein bodies could be more appropriately called vacuoles (Fig. 19). It is interesting to note that protein bodies of wheat endosperm are formed as a result of secretion of proteins into vacuoles (14). The ontogeny of a vacuole in a germinating pea seed might perhaps involve a reverse of the processes of protein body formation.

Needle-like crystalline structures have been observed in many plant tissues by several investigators (6, 43) and were suspected to be a storage form of protein (6). The present author regards such structures as a storage form of protein on the basis of Pronase extraction, and because these structures appeared to become shorter and were less frequently observed as germination progressed.

The occurrence of microbodies (12, 13, 29, 41, 42) and multivesicular bodies (16, 22) in plant tissues has also been previously reported. In the present work, microbody-like organelles were found in pea embryos soaked for more than 8 hr (Figs. 14, 21). Since no crystalline inclusions were found in these organelles, the organelles may be classified as colorless and anucleate microbodies, one of three categories of microbodies (19).

If ER, as has been suggested (19), is involved in the ontogeny of microbodies, it is interesting to note that microbody-like organelles have not been

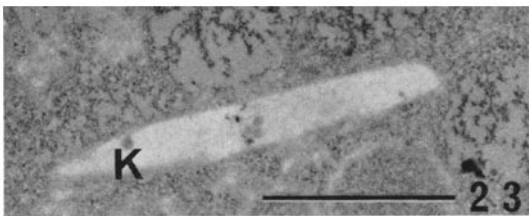


FIGURE 23 Electron micrograph of an 80 min embryo after Pronase extraction. Protein crystal was extracted by Pronase. Scale line = 1μ . $\times 26,000$.

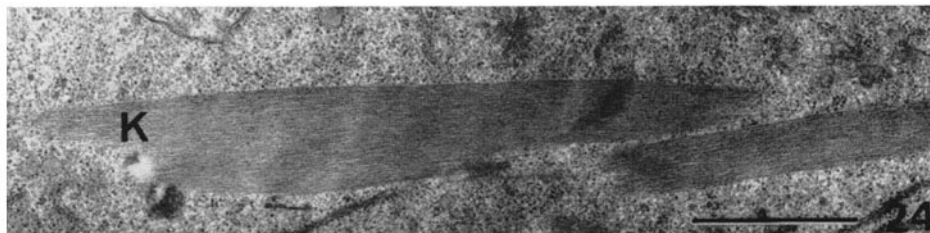


FIGURE 24 Protein crystals in an 8 hr embryo. Scale line = 1μ . $\times 21,800$.

observed in dormant pea embryos where ER is poorly developed. Such failure to find these, however, may reflect that a special histochemical technique is required to demonstrate them in dormant seeds.

It has been suggested (39) that the association of lysosomes, dense bodies, Golgi bodies and their vesicles, microbodies, and multivesicular bodies in animal cells represents an intracellular transport system. Since multivesicular bodies were found in the vicinity of microbody-like organelles (Fig. 21) in germinating pea embryos, it may be that these represent an intracellular transport system.

Dormant pea seeds appeared to retain all the cellular organelles formed during seed maturation, except possibly vacuoles per se and dictyosomes.

As imbibition marks the beginning of germination, the first phase of germination appears to be simply the activation of cellular organelles and development of vacuoles and dictyosomes during the process of germination.

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