THE RELATIONSHIP OF THE AXIS AND THE COTYLEDONS IN GERMINATING SEEDS AND SEEDLINGS OF *PISUM SATIVUM* L.

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**Summary**

Observations recorded extend previous investigations on changes occurring in cotyledons during the development of the seedling of *Pisum sativum* L., and were made in order to further understanding of the complex relation between the axis and cotyledons in germinating pea seeds and seedlings.

The first part of the investigation is concerned with the dependence of the axis on the cotyledons. Plant axes excised from the cotyledons at early stages in seedling development showed little growth in either distilled water or culture solution, even if the root and shoot systems of the seedling were morphologically well developed at the time of excision.

The second part of the investigation is concerned with the control exercised by the axis over changes in the cotyledons after planting. Metabolic and subcellular changes in cotyledons planted after excision from dry seeds and in cotyledons excised and replanted 24 hr and 48 hr after initial planting were compared with those occurring in the cotyledons of developing seedlings. The presence of the axis was not necessary for the conversion of carbohydrate and protein reserves to more soluble forms in rehydrated cotyledons, but was essential in the first 48 hr after planting for completion of the elaborate subcellular organization apparent in the cotyledon cells during the first phase of normal pea seedling development. Nuclei became very lobed only in the presence of the axis. In cotyledons excised during the first 48 hr and replanted there was incomplete development of the endoplasmic reticulum, and mitochondria did not become fully organized.

Such observations on subcellular organization are complementary to data of other workers showing how the axis may control biochemical activity in the cotyledons of developing pea seedlings.

**I. Introduction**

The importance of the cotyledons to the developing pea seedling has been demonstrated in earlier work, and loss of substrates from these storage organs after conversion to transportable forms may be taken as a measure of the dependence of the developing axis on the cotyledons (Bain and Mercer 1966b). There is also an increasing accumulation of biochemical data indicating that some control over metabolic activity in the cotyledons is exerted by the plant axis after pea seeds have been planted. For instance, it has been shown that synthesis of phosphatase does not occur in cotyledons of germinating pea seeds in the absence of the axis (Young 1957a, 1957b; Young and Varner 1959); that mitochondria are "leaky" when

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isolated from cotyledons 24–48 hr after removal of the axis, have a low phosphorylative efficiency, and become completely uncoupled 38–48 hr after excision of the cotyledons (Young et al. 1960); and that excised cotyledons do not take up oxygen 72–92 hr after removal of the axis (Balce 1959, cited by Varner 1961).

Varner, Balce, and Huang (1963), after an extensive investigation of the effect of removing the axis from pea cotyledons at intervals of from 6 to 72 hr after planting, concluded that a heat-labile organic factor is sent from the axis during the first 24–48 hr of germination. Once this signal is received, respiratory metabolism and enzyme activity proceed as in normal germination, even when the axis is subsequently removed; a small piece of attached hypocotyl is sufficient to keep cotyledons healthy for several weeks. Cotyledons lose their colour and turgor in 2–3 days from planting if excised before imbibition is complete; those excised 24–48 hr after planting remain healthy for up to 6 weeks.

Increasing metabolic activity during the first 24–48 hr of germination is accompanied by increasing subcellular organization in the pea cotyledon cell, and during subsequent growth of the axis the changing metabolism of the axis and cotyledons is linked with changing subcellular organization (Bain and Mercer 1966b). Since the axis is known to control the development of certain biochemical and physiological events in the cotyledons, it is possible that it may also have some control over the subcellular organization which develops in these cotyledons during growth of the seedling.

This possibility and the need to know more about the dependence of the axis on the cotyledons stimulated the present investigation. Observations on the influence of the cotyledons over the growth of the plant axis and observations on the influence of the axis over metabolic changes and over subcellular organization in the pea cotyledons after planting are reported in this paper.

II. Materials and Methods

Three phases of morphological development have been defined previously in the pea seedling (Bain and Mercer 1966b). During phase 1 dormancy was broken, the seed germinated (2 days), hydration of the cotyledons was completed, and the radicle lengthened to 40 mm (mainly by cell elongation); part of the carbohydrate and protein reserves were converted to more soluble forms as water was restored to the cotyledons, but these were not lost to the axis. In phase 2 the radicle increased to about 80 mm and secondary root primordia formed, the shoot system expanded from between the cotyledons and broke the ground level; storage reserves were lost from the cotyledons. The seedling became an established plant during phase 3, the cotyledons beginning to deteriorate 10–12 days from planting; loss of storage reserves from the cotyledons continued.

These three phases have been used in the present paper to compare events in normal seedling development with those in the axis following excision of the cotyledons, and with those in the cotyledons following excision of the axis. The cultivar Victory Freezer (Canner's 75 or 99L) was used throughout.
(a) Growth of the Axis following Excision of the Cotyledons

Pea seedlings grown during July in sand kept moist with distilled water were harvested at the end of phase 1 of development (5 days). The roots were then approximately 40 mm long and the shoots were still enclosed by the testa (Plate 1, Fig. 1). The seedlings were divided into two groups: seedlings in one group were left intact and the cotyledons were excised from those in the other group. Half of each of these two groups was then grown for a further 12 days in distilled water, the other half being suspended for the same time in complete culture solution (Hoagland and Arnon 1939). Each seedling was suspended in the medium through a hole bored in a petri dish, the dish forming a lid for the jars containing the solution.

Pea seedlings were also harvested at the end of phase 2 of seedling development (8 days). The shoots had broken the sand surface and the leaves were green; the roots were longer than 80 mm and were developing secondary roots (Plate 1, Fig. 1). These seedlings were treated as above, being grown for a further 9 days in either distilled water or culture solution.

(b) Dependence of the Cotyledons on the Axis

Changes in cotyledons planted after excision from dry seeds were compared with those in the cotyledons of normally developing seedlings to show if physiological and subcellular changes in the cotyledons during germination and seedling establishment were dependent on the presence of the developing axis.

(i) Source of Material.—Whole seeds and cotyledons excised from dry seeds were planted during December in sand wet with distilled water. Samples of 110 cotyledons were taken from each group at intervals after planting. Some of this material was prepared for electron microscopy and the rest was prepared for subsequent analytical determinations. Samples were taken from the excised cotyledons during the first 6 days after planting and cotyledons were sampled from the seedlings during the first 8 days from planting. The seeds had germinated by the second day, i.e. the radicle had broken the testa.

Cotyledons were also excised from seedlings when they had been planted for 24 and 48 hr. These cotyledons were replanted and their subcellular organization examined after a total of 4 days from the first planting. For comparison, similar observations were made on cotyledons of normal seedlings 4 days after planting.

(ii) Analytical and Physiological Data.—In all, 100 cotyledons were taken from the seedlings at each sampling, but the number of excised cotyledons used decreased with time from 100 to 60 per sample, depending on the amount of deterioration in the sample. The average fresh weight, dry weight, and moisture content were found per pair of excised cotyledons from the first to sixth day after planting and per pair of attached cotyledons from the first to the eighth day after planting; starch, total sugar, reducing sugar, total nitrogen, and protein nitrogen content were found per gram dry weight and per pair of these cotyledons as previously described (Bain and Mercer 1966a).
(iii) Preparation of Material for Electron Microscopy.—Pieces of tissue were taken from the inner and outer part of the cotyledons at each sampling and were prepared for electron microscopy as described previously (Bain and Mercer 1966a). Inner and outer tissues were kept separately at each sampling. Osmium tetroxide proved the most satisfactory fixative for cotyledon tissue.

III. Results

(a) Dependence of the Developing Axis on the Cotyledons

Previous analytical data (Bain and Mercer 1966b) showed that the developing axis of the pea seedling was not dependent on the storage reserves of the cotyledons during phase 1 of growth. This phase was characterized by conversion of reserves to more soluble forms, but the soluble nitrogen and sugars were not transported from the cotyledons until phase 2. It seemed likely that the axis was self-supporting during the greater part of phase 1, utilizing the reserves of fat and protein present in the cells of the radicle. These reserves disappeared as the axis expanded. The initial growth of the radicle was due to cell expansion, the root tip becoming meristematic only 4 days after planting.

(i) Growth of the Axis following Excision of the Cotyledons at the End of Phase 1.—Culture solution had a marked effect on the growth of the axes in intact seedlings, the seedlings in culture solution having a larger shoot and root system than those in distilled water alone (Plate 1, Fig. 2). Axes which had been removed from the cotyledons at the end of phase 1 showed no further growth in distilled water and were dead after 17 days; very little growth occurred during the same period in axes placed in culture solution after the cotyledons had been removed from them at the end of phase 1 (Plate 1, Fig. 3).

(ii) Growth of the Axis following Excision of the Cotyledons at the End of Phase 2.—Although the seedling has a well-developed root system and an expanding shoot system at the end of phase 2, axes excised from the cotyledons at this time failed to develop much further in either distilled water or culture solution. Root primordia developed into very short secondary roots and the shoot system showed very little expansion in the 9 days after removal of the cotyledons (Plate 1, Fig. 3). The beneficial effects of the cotyledons plus culture solution on the growth of the axis in normal seedlings were very obvious during phase 3 of seedling development (Plate 1, Fig. 2).

(b) Dependence of the Cotyledons on the Axis

Comparative observations of changes in the cotyledons of normal seedlings and those in cotyledons excised prior to planting could be carried out for only about 1 week after planting, because the excised cotyledons were subject to infection. These seedlings, being planted in December, were grown at higher temperatures than were those in the previous experiments and those described by Bain and Mercer (1966b). Consequently the morphological phases established previously for seed development were completed more quickly in this part of the present investigation. Phase 1 lasted 4 instead of 5 days and phase 2 was completed after 6 instead of 8 days.
(i) Analytical and Physiological Data

Analytical and physiological data for the cotyledons from each treatment are given in Figure 1.

Fig. 1.—Changes in fresh weight (○) and dry weight (●) expressed as grams per pair of cotyledons, and changes in total nitrogen (△), protein nitrogen (▲), starch (□), and sugar (■) expressed as milligrams per pair of cotyledons. Data for the three morphological phases in normal seedling development are compared with data for cotyledons which were excised from the dry seed prior to planting.

The excised cotyledons absorbed more water than did the normal cotyledons, up to the fifth day. There was little change in dry weight up to the fourth day in either the attached or the excised cotyledons, but growth of the axis in phase 2 of seedling development then caused a loss in dry weight in the attached cotyledons. The analytical data for cotyledons indicated that the presence of the axis was not necessary to initiate the conversion of reserve carbohydrate and protein to more soluble forms following breaking of dormancy. Starch appeared to break down at a
### Table 1

**Subcellular Organization in the Cotyledon Cells at the End of 4 Days from Planting**

<table>
<thead>
<tr>
<th>Cell Structure</th>
<th>Cotyledons of Seedlings at End of Phase 1 of Development (4 days)</th>
<th>Cotyledons Excised from Dry Seeds prior to Planting</th>
<th>Cotyledons Excised from Seeds 24 hr after Planting and Replanted for a further 3 Days</th>
<th>Cotyledons Excised from Seeds 48 hr after Planting and Replanted for a further 2 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondria</td>
<td>Structure fully organized (Plate 2, Fig. 2)</td>
<td>Recognized from their size and shape, but internal structure incomplete (Plate 2, Fig. 4)</td>
<td>Cristae structure partly organized (Plate 3, Fig. 1)</td>
<td>Structure fully organized (Plate 3, Fig. 3)</td>
</tr>
<tr>
<td>Endoplasmic reticulum</td>
<td>A system of long, paired, parallel, and smooth membranes (Plate 2, Fig. 1)</td>
<td>—</td>
<td>—</td>
<td>A system of long, paired, parallel, and smooth membranes (Plate 3, Fig. 2)</td>
</tr>
<tr>
<td>Vesicles in cytoplasm</td>
<td>Vesicles formed during first 48 hr had fused to form the endoplasmic reticulum</td>
<td>Small vesicles throughout cytoplasm (Plate 2, Fig. 3)</td>
<td>Vesicles increased in number and size from the second to the fourth day, but did not fuse linearly to form the endoplasmic reticulum of normal seedling development (Plate 3, Fig. 1)</td>
<td>Vesicles formed during the first 48 hr had fused to form the endoplasmic reticulum</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Nuclei very lobed, indicating a very active metabolic state (Bain and Mercer 1966b)</td>
<td>Nuclei only slightly lobed, in contrast to previous condition (Plate 4, Fig. 2)</td>
<td>No nuclear pattern observed</td>
<td>Nuclei very lobed, structure similar to that in cells of attached cotyledons (Plate 4, Fig. 1)</td>
</tr>
<tr>
<td>Storage reserves</td>
<td>Light microscope and histochemical observations showed that starch, protein, and fat reserves were plentiful in the cotyledons in all treatments. Their form was similar to that found in previous electron micrographs (Bain and Mercer 1966b) and this did not alter in the first 4 days after planting</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
faster rate in the excised cotyledons, but increase in sugar content was comparable in both treatments. Changes in total and protein nitrogen were parallel in the presence or absence of the axis in the first four days after planting, and thereafter loss was greatest in the attached cotyledons.

(ii) Subcellular Organization

The subcellular organization observed in the various treatments after a total of 4 days from planting is summarized in Table 1.

(1) Attached Cotyledons.—Subcellular organization observed in these cotyledons during phases 1 and 2 of seedling development was similar to that already described (Bain and Mercer 1966a), except that the changes occurred more rapidly at the higher temperatures of planting.

The subcellular organization after germination for 24 hr was very similar to that of a cell approaching dormancy (Bain and Mercer 1966a); mitochondria (identified by size, shape, and incomplete cristae structure) and an occasional, ill-defined Golgi body could be identified in the cytoplasm, which was undifferentiated except for a few small vesicles. Previously identified starch, protein, and fat reserves were obvious in the cytoplasm, the protein and some fat deposits being enclosed by a distinct membrane.

Two days after planting, clusters of small vesicles had formed in the cytoplasm. They were usually observed in close proximity to fat deposits. Mitochondrial structure was fully organized and Golgi bodies were now more distinct and more frequent. The small vesicles had increased in number and had begun to fuse together linearly by the third day. By then nuclei were becoming lobed and had prominent nucleoli, and only a few ribosomes were detected in the cytoplasm. Little change was seen in the form of the storage reserves.

The fusing cytoplasmic vesicles had elongated by the fourth day (end of phase 1) and had formed an elaborate endoplasmic reticulum system. This system was made up of long, paired, smooth membranes and penetrated the cytoplasm (Plate 2, Fig. 1). Ribosomes had increased in the cytoplasmic matrix and nuclei were much enlarged and very lobed. Fully organized mitochondria were numerous (Plate 2, Fig. 2). The form of the storage reserves was unaltered, but their amount had decreased.

This elaborate subcellular organization was short-lived, and broke down again during phase 2 of seedling development. The endoplasmic reticulum became a series of vesicles, mitochondria and Golgi bodies became disorganized, and nuclei became less lobed. Starch and protein content showed a further decrease during phase 2 and fat content increased as the membrane system of the cell became disorganized.

(2) Excised Cotyledons.—Subcellular organization in cotyledons excised from the dry seed and then planted was similar to that in the attached cotyledons for the first 2 days after planting, except that the mitochondria were incompletely organized. Vesicles continued to form in the cytoplasm during the next few days, but there was no development of endoplasmic reticulum made up of long, paired, and parallel membranes after the fourth day of planting (Plate 2, Fig. 3). Mitochondria were recognizable then, but their organization was incomplete (Plate 2, Fig. 4). Nuclei
were either rounded or slightly lobed (Plate 4, Fig. 2), their form being in marked contrast to that of nuclei in the cotyledons of seedlings. The tissue became senescent after this time, vacuoles developing from small vesicles in the cytoplasm.

The subcellular organization in cotyledons excised 48 hr after planting and replanted for 2 more days (Plate 3, Figs. 2 and 3) resembled that observed in the attached cotyledons at the end of phase 1 in seedling growth (Plate 2, Figs. 1 and 2); the vesicles in the cytoplasm had fused linearly to form an elaborate endoplasmic reticulum and the mitochondria and Golgi bodies appeared to be fully organized. Nuclei were very lobed (Plate 4, Fig. 1). The organization began to break down 4 days after initial planting, vacuoles forming in the cytoplasm and the mitochondria becoming disorganized.

Subcellular organization in cotyledons excised 24 hr from planting and replanted for 3 further days was intermediate between that found in cotyledons excised after 48 hr and that in cotyledons excised from the dry seeds and planted for 4 days. Vesicles continued to form in the cytoplasm of these cotyledon cells after the second day, but only a small proportion of them fused to form a membranous system (Plate 3, Fig. 1). The elaborate endoplasmic reticulum found in the cotyledons of seedlings at the end of phase 1 of development, and in cotyledons excised 48 hr after initial planting and replanted for a further 2 days, was not developed; the organization of mitochondria and Golgi bodies was incomplete. Subcellular structure began to break down at the end of 4 days of planting; vacuoles, characteristic of senescence, became very conspicuous in the cytoplasm.

**IV. DISCUSSION**

The relation of the cotyledons and the axis to each other is very complex and must necessarily involve a study of the interaction of growth substances as well as the availability of reserve materials in the cotyledons and their depletion by the axis.

The present data, though not exhaustive in relation to the dependence of the seedling on the cotyledons, have established the degree of dependence of the plant axis on the cotyledons during the three morphological phases of development established previously for the growth of a pea seedling (Bain and Mercer 1966b).

Analytical data show that the pea axis is not dependent on the reserve material of the cotyledons during phase 1 of seedling growth, i.e. up to the time when the radicle is approximately 40 mm long and the shoot system not yet expanded. The plentiful reserves of protein and fat (both observed in the radicle of the seed approaching dormancy) must be sufficient for the early development of the axis. Initial growth of the axis is by cell expansion, cell division only becoming active in the radicle towards the end of phase 1 of seedling growth. Phase 1 is characterized by the conversion of reserve carbohydrate and protein material to more soluble forms in readiness for transport to the developing axis, but these are not lost from the cotyledons during that time.

It is to be expected that the growth of the axis would be dependent on the cotyledons during phase 2, i.e. during the development of the shoot system and further development of the root system. This is shown to be so by the fact that the axes excised at the end of phase 1 do not continue to grow in the culture solution.
At the end of phase 2, when the shoot system of the seedling is expanded above the surface and the root system has developed secondary root primordia, it might be expected that the plant axis would cease to be dependent entirely on the cotyledons for growth. However, the small amount of growth observed in the axis when the cotyledons were excised at the end of phase 2 indicates that this is not so.

The fact that the greatest loss of reserve material occurs from the cotyledons during phase 3 of seedling growth, when the root and shoot system are completely developed (i.e. up to 3 weeks from planting), indicates that the young plant is dependent on the seed reserves long after morphological development would indicate. The cotyledons alone, however, do not support maximum growth of the seedling after its earliest phase of development; this is evident from the increased growth of the seedlings in culture solution over that of seedlings grown in distilled water alone.

The addition of substrates and co-factors to the culture solution is necessary before the dependence of the axis on the cotyledons can be fully elucidated. In preliminary experiments, sucrose added to the culture solution did not increase the growth of the excised axis appreciably, suggesting that the cotyledons supply more than a simple carbon source to the axis.

Some biochemical evidence is available on the materials supplied to the developing seedling axis by storage tissue in the seed. Okamota (1962) showed that half of the divalent cations accumulating in the seedling of *Vigna sesquipedalis* came from the cotyledons; 90% of the potassium in the cotyledons is transported, but practically all the calcium remains in the cotyledons. Osawa and Oota (1953) and Oota, Fujii, and Osawa (1953) showed that nucleic acids are transported from bean cotyledons in the first 5 days from planting. Oota and Takata (1959) concluded that ribonucleic acid is transported from bean cotyledons in an intact form, the loss from the cotyledons being accounted for by an increase in amount in the seedling axis. Barker and Douglas (1960) suggested that ribonucleic acid is degraded by ribonuclease in the cotyledons of germinating seeds and that polynucleotides are transported to the axis. Ledoux, Galand, and Huart (1962), who followed the distribution of nucleic acids and protein synthesis in the component parts of the barley seedling, showed that ribonucleic acid disappeared from the endosperm in the first 3 days of germination while deoxyribonucleic acid remained constant, but that the former accumulated in the shoot and root of the seedling. They concluded that the function of the storage tissue is to supply undegraded macromolecules to other parts of the seedling. Changes in the ribonucleic acid content of the various parts of developing seedlings have also been reviewed by Oota (1964).

The present observations on excised pea cotyledons indicate that the presence of the axis is necessary for complete subcellular organization such as is found in cotyledon cells at the end of phase 1 in pea seedling development; also, that only part of this organization occurs if the axis is removed 24 hr after planting, and that complete organization results only if the axis is not detached within 24–48 hr of initial planting.

Formation of membranes in the cotyledon cells following planting appears to be related to the increasing water content of the tissue, but the development of the structural organization from these membranes appears to be controlled by the
presence of the plant axis. Mitochondria, Golgi bodies, and the endoplasmic reticulum show complete organization at the end of phase 1 only if the plant axis remains attached to the cotyledons during the 24-48 hr period after planting. If the cotyledons are removed sooner, the degree of subcellular organization increases with increasing time after planting and with increasing time prior to excision.

The period between the 24th and 48th hr after planting of the seed appears to be a very important one in the relation between the axis and the cotyledons, both from the subcellular and metabolic aspect. Varner, Balce, and Huang (1963) showed the importance of the presence of the axis during the first 24-48 hr of germination, the stimulus transmitted from it during that time being held responsible for regulating further metabolic activity in the cotyledons. The decline in activity in mitochondria isolated from cotyledons excised prior to 48 hr from planting (Young et al. 1960) can be related to the incomplete reorganization of mitochondria seen in the present observations when the axis was removed before the seed had been planted for 24 hr.

The development of the elaborate reticulum in the cotyledon cells at the end of phase 1 in normal seedling development, and its subsequent breakdown, presented a problem in previous observations (Bain and Mercer 1966b). It was thought that the endoplasmic reticulum may have been important in transporting soluble reserves through the cotyledons to the axis, its development coinciding with the beginning of the growth of the axis; but its immediate breakdown at the beginning of phase 2 in seedling development made this function seem unlikely. It was postulated, therefore, that the endoplasmic reticulum was a storage system for the sugar that accumulated in the cells as starch was broken down, and that its membranes formed as water content increased. The formation of an incomplete endoplasmic reticulum in cotyledons excised and replanted 24 hr from initial planting indicates that the axis is important in controlling the development of subcellular structure in the cotyledon cells. The development of such a system in cotyledons excised 48 hr after planting and replanted for a period equivalent to the time of phase 1 in seedling development suggests that this system might have a storage function.

Level of water in the pea cotyledon cells, rather than the relation between axis and cotyledon, appears to control the breakdown of carbohydrates and protein storage reserves. Water level possibly controls the formation of "non-oriented" membranes in the cytoplasm, but the axis appears to control the organization of subcellular structure, transforming the non-oriented membranes into organized systems, e.g. the endoplasmic reticulum and the cristae structure of the mitochondria. It would be of considerable interest to know whether the heat-labile factor postulated by Varner, Balce, and Huang (1963) as being responsible for regulating the recovery of metabolic activity during germination, also controls the recovery of subcellular organization.

V. Acknowledgments

We wish to thank Mr. J. Smydzuk for analytical data on prepared material; also Mr. P. R. Maguire for the photographs reproduced in Plate 1.
VI. References


BALCE, H. V. (1959).—Factors controlling cellular aging in plant tissues. M.S. Thesis, Ohio State University, Columbus, Ohio. (Cited by Varner 1961.)


Explanation of Plates 1–4

All figures in Plates 2–4 are electron micrographs of pea cotyledon tissue which was fixed in buffered 1% osmium tetroxide, stained with uranyl acetate, embedded in Araldite, and sectioned

Plate 1

Fig. 1.—Morphological development of the pea seedling at the end of phases 1 (5 days) and 2 (8 days). At the end of phase 1 the radicle is about 40 mm long and the shoot is still enclosed within the split testa of the seed. At the end of phase 2 the secondary root primordia are developed and the shoot system has expanded above the surface. ×1·2.

Fig. 2.—Pea seedlings grown in sand wet with distilled water for 5 days (end of phase 1) followed by distilled water (A) or culture solution (B) for a further 12 days. ×0·3.

Fig. 3.—Comparison of plant axes (A and B) excised from seeds growing in sand wet with distilled water for 5 days (end of phase 1 of seedling development) with axes removed from similar seedlings (C and D) at the end of 8 days after planting (end of phase 2 of seedling development). A and C placed in distilled water for 12 and 9 days, respectively, after excision. B and D placed in culture solution for 12 and 9 days, respectively, after excision. ×0·6.
Plate 2

Fig. 1.—Subcellular organization found in a cell of a cotyledon of a seedling at the end of phase 1 in its morphological development (4 days after planting). The endoplasmic reticulum (ER) is made up of long, paired, and parallel membranes, forming a network in the cytoplasm (Cyt). Fat deposits (F), protein material (P), mitochondria (M), and the cell wall (CW) are shown. ×15,000.

Fig. 2.—Detail of mitochondria (M) in a cell of a cotyledon of a seedling at the end of phase 1 in its morphological development (4 days after planting). ×30,000.

Fig. 3.—Subcellular organization in a cell of a cotyledon which was excised from the dry seed and planted for 4 days (time comparable to the end of phase 1 in seedling development). Vesicles (V) have formed in the cytoplasm (Cyt), but there is no fusion to form an elaborate endoplasmic reticulum as in normal seedling development (compare Plate 2, Fig. 1). Mitochondria (M) are incompletely organized. Fat deposits (F) are frequent in the cytoplasm. ×15,000.

Fig. 4.—Detail of mitochondria (M) in a cell of a cotyledon which was excised from the dry seed and planted for 4 days (time comparable to the end of phase 1 in seedling development). ×40,000.

Plate 3

Fig. 1.—Subcellular organization in a cell of a cotyledon which was excised from a seed after germination for 24 hr and replanted for 3 days. Vesicles (V) have continued to form in the cytoplasm (Cyt), but there has only been a limited amount of fusion of these vesicles to form an "endoplasmic reticulum" ("ER"). Mitochondria (M) possibly show more organization than those in Plate 2, Figure 4. Protein reserves (P) and fat deposits (F) are shown. ×25,000.

Fig. 2.—Subcellular organization in a cell of a cotyledon which was excised from a seed after germination for 48 hr and replanted for 2 days. An elaborate endoplasmic reticulum (ER) of long, paired, and parallel membranes has formed in the cytoplasm (Cyt). This membrane system is similar to that formed in the cotyledons of a seedling 4 days after planting (compare Plate 2, Fig. 1). ×30,000.

Fig. 3.—Detail of the structure of mitochondria (M) in a cell of a cotyledon which was excised from a seed after germination for 48 hr and replanted for 2 days. The structure of these mitochondria (M) is comparable to that of mitochondria in cotyledons of a seedling 4 days after planting (compare Plate 2, Fig. 2). The cytoplasm (Cyt) and the endoplasmic reticulum (ER) are indicated. ×30,000.

Plate 4

Fig. 1.—Shows the marked lobing of portion of a nucleus (N) in a cell of a cotyledon which was excised from the seedling after germination for 48 hr and replanted for 2 days. The cytoplasm (Cyt) and a mitochondrion (M) are shown. ×15,000.

Fig. 2.—Shows the structure of a nucleus (N) in a cell of a cotyledon which was excised from a dry seed and then planted for 4 days. This nucleus shows very little lobing when compared to the structure of the nucleus in Plate 4, Figure 1. A nucleolus (Nuc) is prominent in the nucleus. Starch grains (SG) are shown in the disorganized cytoplasm (Cyt). ×3750.
COTYLEDON-AXIS RELATIONS IN PEA SEEDLINGS

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