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Embryogeny of *Phaseolus coccineus*: Growth and Microanatomy

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With 26 Figures

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Summary

During early embryogeny, the development of the suspensor is rapid both in terms of size and fresh weight; structural differentiation can be observed as early as the proembryo stage with the formation of wall ingrowths. Ingrowths first appear in the outer wall of the suspensor cells adjacent to the integumentary tapetum, soon ingrowths begin to form in the inner suspensor cells as well. A basal-terminal gradation in nuclear size exists, with the largest nuclei in the basal suspensor cells. Cytologically, the suspensor cells appear to be very active, especially when the embryo reaches heart stage. Initially, the development of the embryo proper lags behind the suspensor, but its size and fresh weight increase rapidly as development proceeds. The volume of the liquid endosperm rises most rapidly during the late heart stage; and it is absorbed soon after. A cellular endospermic sheath surrounds the embryo, separating it from the liquid endosperm. Structural differentiation also occurs in the cellular endosperm cells with the formation of wall ingrowths in those cells that abut directly onto the integumentary tapetum. Both the suspensor and the cellular endosperm appear to remain active through the maturation of the seed. Storage bodies are formed in the cotyledons as well as in the embryonic axis. In the suspensor and the cellular endosperm, starch grains and lipid bodies can be found at the maturation stage.

1. Introduction

In recent years, there has been renewed interest in the study of plant embryo development because of the potential value of *in vitro* embryo culture as a tool in plant breeding, as well as for its use in genetic and developmental studies. However, our understanding of *in vivo* embryo development is far from complete, and knowledge of the requirements for the growth of isolated embryos is limited. While information concerning the physical and chemical environment of the embryo is available for only a few species (RIJVEN 1952, RYCZKOWSKI 1960, 1974, SMITH 1971, 1973), there is a more extensive literature both classical and recent microscopic studies (for reviews see MAHESHWARI 1950, WARDLAW 1965, STEEVES and SUSSEX 1972, JENSEN 1974) which has

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defined the morphological environment of the embryo in a large number of species. These studies have contributed significantly to our understanding of embryo development.

During the past decade a greater understanding of early events in Angiosperm embryogeny has been made possible by ultrastructural (CLUTTER and SUSSEX

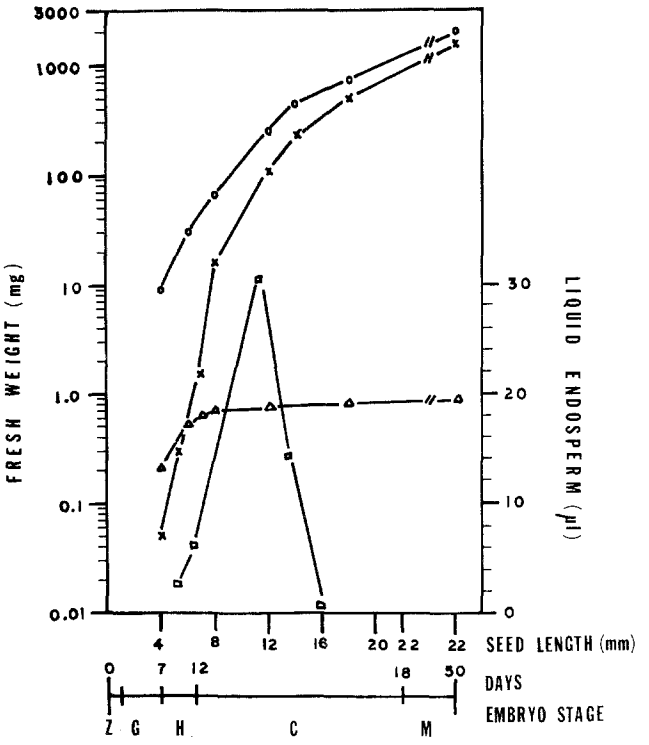

Fig. 1. Changes in the fresh weight of the seed (—·O—) and its component parts, *i.e.*, the embryo proper (—×—) and the suspensor (—△—); and the changes in the volume of the liquid endosperm (—□—) through development. Embryo stage: Z = zygote, G = globular, H = heart, C = cotyledon, and M = maturation

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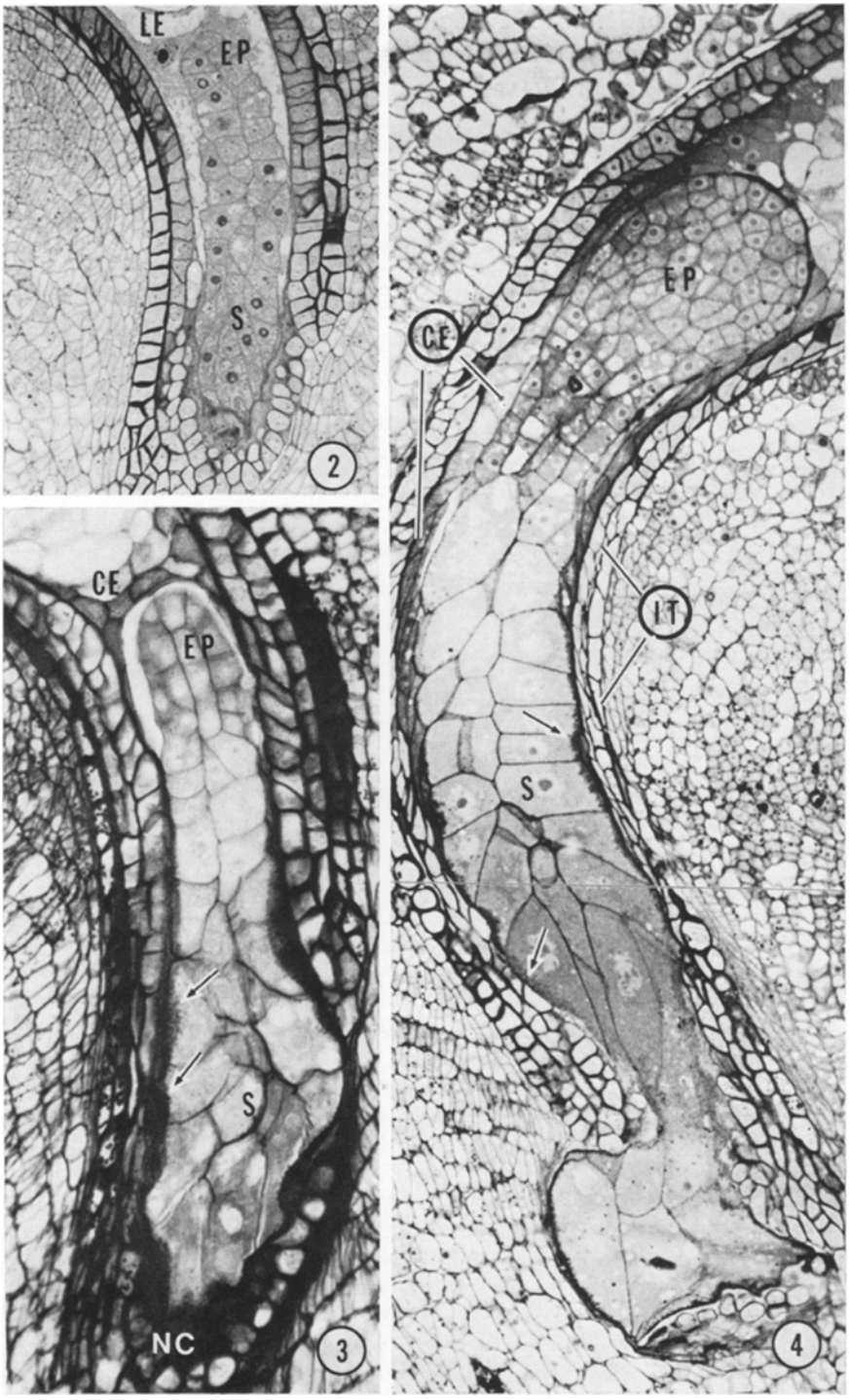
Plate 1. Figs. 2–4. Developing embryos of *Phaseolus coccineus*

Fig. 2. Median longitudinal section of an early proembryo showing very little structural differentiation between the suspensor (*S*) and the embryo proper (*EP*). The embryo appears to be in direct contact with the liquid endosperm (*LE*). ×350

Fig. 3. Median longitudinal section of a late proembryo. The suspensor (*S*) shows structural differentiation in the form of wall ingrowths (arrows). The basal region of the suspensor has become slightly enlarged and is pressed against the remains of the nucellar cap (*NC*). The cells in the embryo proper (*EP*) are smaller and arranged in a regular fashion. The embryo is separated from the liquid endosperm by layers of cellular endosperm (*CE*). ×600

Fig. 4. Longitudinal section of an early globular embryo. The embryo proper (*EP*) is undifferentiated at this time. The relatively advanced development of the suspensor (*S*) is apparent from the ingrowths (arrows) along the wall adjacent to the integumentary tapetum (*IT*) and the cellular endosperm (*CE*). ×700



Figs. 2–4

1968, SCHULZ and JENSEN 1969, SCHNEPF and NAGL 1970, PONZI and PIZZOLONGO 1972, NEWCOMB 1973, NEWCOMB and FOWKE 1974, SIMONCIOLI 1974) and biochemical studies (WALBOT *et al.* 1972 a, SUSSEX *et al.* 1973, CLUTTER *et al.* 1974). They suggest that during early embryogeny, the suspensor plays an active role in embryo development rather than serving merely to connect the embryo proper to the maternal tissues. Furthermore, *in vitro* culture experiments have shown that the growth of *Eruca* embryos is stimulated by the presence of the suspensor at the heart stage of embryo development (CORSI 1972). These data support the idea that the suspensor may be involved in the absorption and transport of nutrients to the embryo proper (SCHULZ and JENSEN 1969; NAGL 1974; NEWCOMB and FOWKE 1974; SIMONCIOLO 1974). Recently, gibberellins have been found in the suspensor of *Phaseolus coccineus* (ALPI *et al.* 1975), implying that the suspensor may be a site of hormone synthesis as well (CORSI 1972; NAGL 1974; ALPI *et al.* 1975).

The work presented here characterizes, at the light microscope level, the changes in morphology which the embryo undergoes during embryogeny, with emphasis being placed on the early stages. In this paper particular attention will be paid to the development of the suspensor in order to better define its role during early embryogeny. These structural changes, when considered in relation to our present knowledge of seed development, will serve as the background for future experimental studies of this species.

2. Materials and Methods

2.1. Plants

Embryos were obtained from developing seeds of *Phaseolus coccineus* plants which were grown in Marsh Garden at Yale University, New Haven, Connecticut, during the summers of 1973–1976.

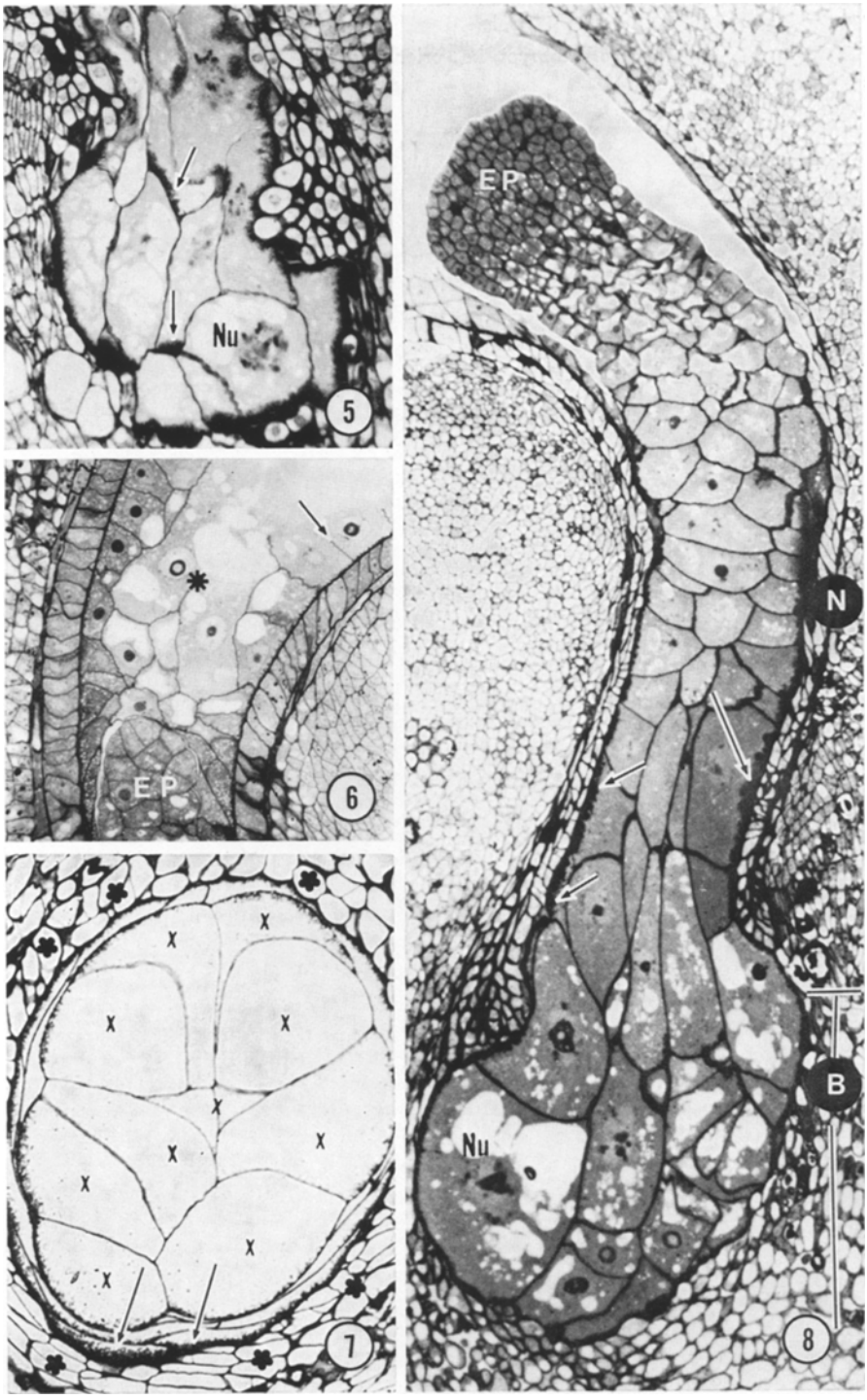
Plate 2

Fig. 5. Extensive vacuolation starts in the basal suspensor cells at the globular stage. Additional wall ingrowths (arrows) are formed in the inner suspensor cells; they are formed predominantly on the basal side of the cell. Polytene chromosomes are visible in the nuclei (*Nu*) of the suspensor cells. ×400

Fig. 6. The formation of the cellular endosperm. Cellular endosperm is formed near the embryo proper (*EP*); its cell wall is initiated either directly from the embryo sac wall (arrow) or from other cellular endosperm cells (*). ×400

Fig. 7. Transverse section of the suspensor at the neck region showing the position of the wall ingrowths (arrows) of the cellular endosperm. These ingrowths are formed next to the integumentary tapetum (*) and are not formed on the side adjacent to the suspensor cells (X). This section is stained for total carbohydrates with the PAS stain only, to enhance the detection of wall ingrowths. ×550

Fig. 8. Median longitudinal section of an early heart embryo. At this stage, the cytoplasm of the cells in the embryo proper (*EP*) are deeply stained. In the suspensor, wall ingrowths (arrows) are well developed and a basal-terminal gradation in nuclear size can be seen with the largest nuclei (*Nu*) in the basal suspensor cells. Different regions of the suspensor, *i.e.*, the basal region (*B*) and the neck region (*N*) are also outlined. ×420



Figs. 5–8

2.2. *Histological Methods*

For the examination of small embryos, *i.e.*, prior to the heart stage of embryo development, most of the seeds were fixed using the following schedule. Developing seeds were removed from the pods, and a small cut was made near the chalazal end of each seed in order to facilitate the penetration of fixatives and the embedding medium. The seeds were then fixed immediately in a 3% glutaraldehyde solution buffered with 0.08 M cacodylate buffer at pH 7.2. The tissues were fixed at room temperature for one hour; transferred to ice for another three hours; washed several times in 0.08 M cacodylate buffer; and post-fixed in 2% osmium tetroxide using the same buffer. The tissues were dehydrated through a graded acetone series and embedded in Epon-Araldite mixture (mixture 1 of MOLLENHAUER 1964). All sections were cut with glass knives on the Reichert Om-U 2 ultramicrotome. The sections were first pre-treated with acidified H_2O_2 for two minutes (POOL 1973) before staining with PAS-Toluidine Blue O (FEDER and O'BRIEN 1968).
For another series of developing seeds, particularly those with large embryos (*i.e.*, beyond heart stage of embryo development), the glycol methacrylate technique was used. Whole seeds or embryos were fixed in a non-buffered 10% acrolein solution at 4 °C, then dehydrated and embedded in glycol methacrylate according to the general techniques of FEDER and O'BRIEN (1968). Serial 2 μm-thick sections were cut with glass knives with the Sorvall MT-1 ultramicrotome. The sections were stained with PAS-Toluidine Blue O (FEDER and O'BRIEN 1968).

2.3. *Cytochemical Staining of Total Proteins and Polysaccharides*

Tissues for cytochemical studies were prepared as previously described and only glycol methacrylate sections were used. Sections were stained for total proteins with aniline blue black (FISHER 1969) and for polysaccharides with periodic acid-Schiff (FEDER and O'BRIEN 1968).

2.4. *Clearing of Vascular Tissues*

The vascular pattern in the seed coat of the developing seeds was revealed by treatment with lactic acid (SACHS 1968). The seed coat was immersed in lactic acid which was heated to 100 °C once and stored at room temperature for a day before examination of the seed coat.

2.5. *Growth Measurements*

Embryos and suspensor were dissected from seeds of selected lengths and weighed on a Mettler M-5 microbalance. The volume of the endospermic fluid of seeds was measured using a Hamilton microliter syringe. Each determination on Fig. 1 is an average value of at least 10 individuals.

2.6. *Terminology*

The term *embryo* refers to the products of the zygote, *i.e.*, the *suspensor* and the diploid organogenetic part of the embryo or *embryo proper* (ESAU 1965). The suspensor is arbitrarily divided into two regions for the ease of description. The basal region of the suspensor refers to the enlarged region which is attached to the seed coat, the narrower upper portion is termed the *neck* region (see Fig. 8).
The epidermis of the inner integument located next to the embryo sac is termed the *integumentary tapetum* (ESAU 1965).

3. Results

The general post-fertilization development of *P. coccineus* has been described by WALBOT *et al.* (1972 b) and BRADY (1972). The successive developmental stages of the embryo, *i.e.*, proembryo, globular, heart, cotyledon and maturation, can be identified by pod and seed length. These stages will be used in the following description of embryo development.

3.1. Growth Measurements

Fig. 1 summarizes the changes in the fresh weight of the seed and its component parts. During early development, the seed coat constitutes the major portion of the fresh weight of the seed, and until day six, the suspensor is heavier than the embryo proper. On the sixth day of development the embryo proper weighs more than the suspensor. Subsequently the weight of the suspensor increases at a slower rate while the fresh weight of the embryo proper continues to increase rapidly.

The volume of the liquid endosperm increases very rapidly at late heart stage and reaches a maximum at early cotyledon stage. Later, the liquid endosperm is quickly absorbed (Fig. 1).

3.2. Anatomical Changes in the Embryo at Different Stages of Development

3.2.1. Proembryo Stage

After fertilization, the zygote divides periclinally resulting in a large basal cell and a smaller terminal cell (BRADY 1972). Further cell divisions of these two cells result in the formation of a filamentous embryo (Fig. 2). There is little structural differentiation at this time; the general cytological features of the cells derived from the terminal and the basal cell appear to be the same (Fig. 2). Serial sections reveal a prominent nucleolus in the nucleus of every cell (Fig. 2).

Just prior to the globular stage of embryo development, the suspensor cells have differentiated so that they are distinct from the embryo proper. The basal region has enlarged and is pressed against the remains of the nucellar cap of the embryo sac (Fig. 3). Judging from their asymmetric shape and non-uniform structure (Figs. 2, 3, 4, and 8), cells in the suspensor appear to undergo a non-synchronous pattern of cell division and differentiation. Cells in the embryo proper, however, are smaller and arranged in a regular fashion (Fig. 3). Cell division in the basal region of the suspensor, has most likely ceased, as polytene chromosomes have been observed and their DNA content measured in the basal suspensor cells at this time (BRADY 1972, 1973). Furthermore, the cell number in the basal region of the suspensor is approximately the same from this stage onward (compare Figs. 2, 3, and 8). Any further growth of the suspensor in this region is by cell enlargement.

Cytologically, one of the major characteristics of the suspensor is the presence of wall ingrowths. These ingrowths can be seen in the basal suspensor cells at the late proembryo stage, primarily on the wall adjacent to the integumentary tapetum (Fig. 3). Vacuolation also begins in the basal suspensor cells just prior to the globular stage.

Early in development the proembryo seems to be in direct contact with the liquid endosperm (Fig. 2). But soon, it is clearly separated from the liquid endosperm by a layer of cellular endosperm (Fig. 3).

3.2.2. Globular Stage

Continued cell divisions in the embryo proper result in a globular shaped embryo. The cells in the embryo proper are cytologically similar to each other; they are very small in size and the cytoplasm of these cells is very dense with a few small vacuoles (Fig. 4).

In the suspensor, cell division continues and is mainly confined to the terminal neck region of the suspensor near the embryo proper (BRADY 1972). The basal region of the suspensor gradually protrudes into the maternal tissue, in the micropylar region of the seed coat, having penetrated about 200 μm into the maternal tissue by the early cotyledon stage. Several interesting cytological features are observed at this stage. As the embryo approaches heart stage, extensive vacuolation occurs in the cytoplasm of the basal suspensor cells (Fig. 5) while the remaining suspensor cells are highly cytoplasmic. The nuclei of the suspensor cells are larger than those of the embryo proper (Fig. 4) and, portions of polytene chromosomes are visible (Fig. 5). Wall ingrowths are well developed at this stage with the formation of the ingrowths proceeding from the basal cells towards the embryo proper (Fig. 4). They first appear in the outer wall of the suspensor cells adjacent to the integumentary tapetum (Fig. 4). Soon wall ingrowths begin to form in the inner suspensor cells as well; these ingrowths are formed predominantly on the basal side of the cell (Fig. 5).

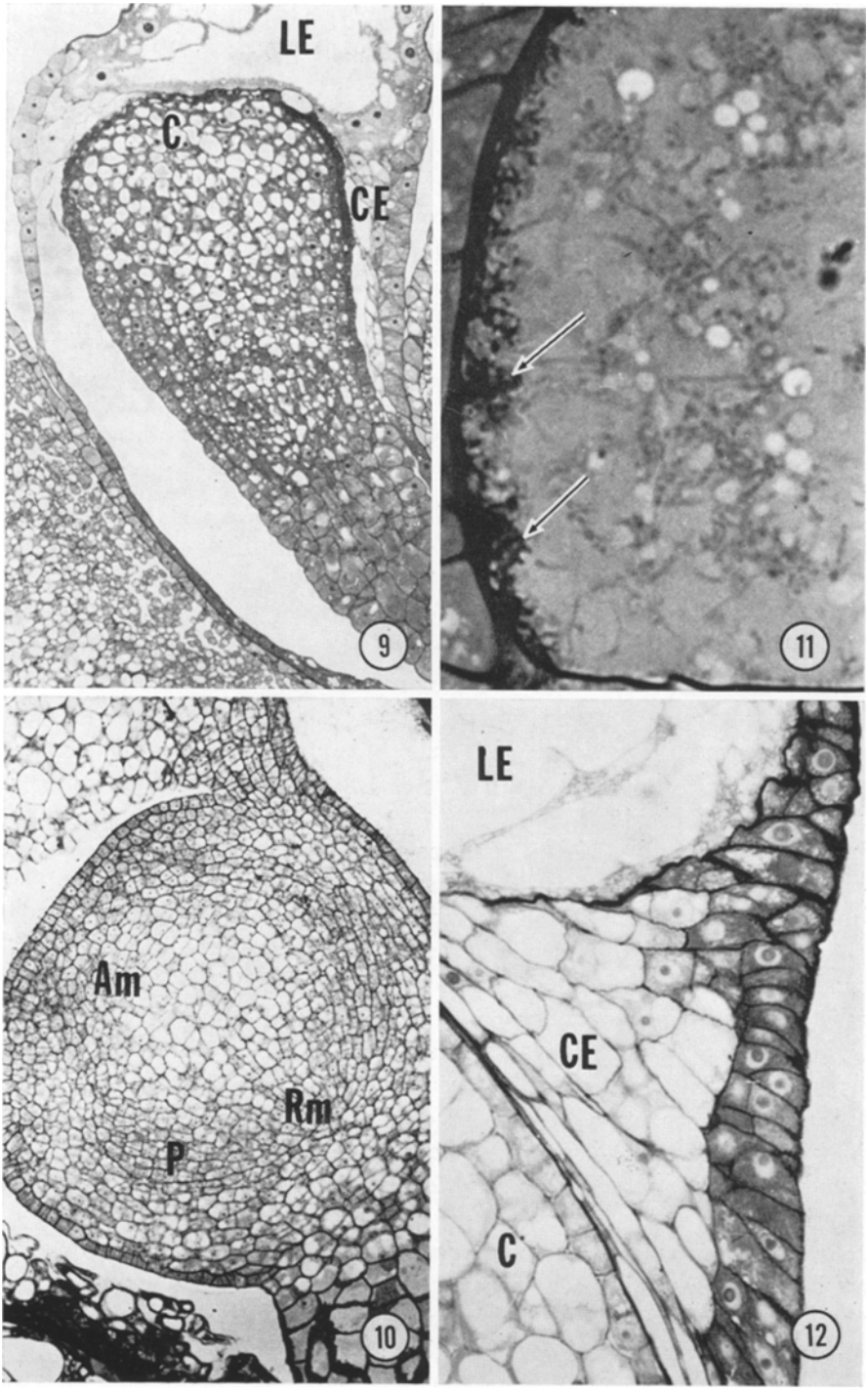
Plate 3. Figs. 9—12. Some cytological features of the heart stage embryo.

Fig. 9. Vacuolation of the cells of the embryo proper at mid-heart stage, especially in the cotyledon primordia (*C*). The embryo is separated from the liquid endosperm (*LE*) by the cellular endosperm (*CE*). ×350

Fig. 10. Median longitudinal section of the developing embryonic axis at late heart stage showing future meristems of the shoot (*Am*) and the root (*Rm*). Procambium (*P*) also starts to differentiate at this time. ×180

Fig. 11. Suspensor cell at heart stage showing extremely well developed wall ingrowths (arrows), dense cytoplasm and numerous cellular organelles. ×1,800

Fig. 12. Longitudinal section through the developing cotyledon (*C*) showing the cellular endosperm (*CE*) at the junction of the expanding cotyledon and the liquid endosperm (*LE*). ×450



Figs. 9–12

The cellular endosperm surrounding the embryo has a very dense cytoplasm as indicated by the high intensity staining of the cytoplasm (Figs. 4 and 6). Cellularization of the endosperm occurs mainly near the developing embryo (Figs. 4 and 6). Two methods of wall formation have been observed. New wall is first laid down by the furrowing method (ESAU 1965); it appears to be initiated either directly from embryo sac wall or from other cellular endosperm (Fig. 6). After the formation of the cellular endosperm, normal cell division occurs and the new wall is formed from the phragmoplast and cell plate (Ultrastructural observation, YEUNG, unpublished). Soon after the beginning of the cellularization of the endosperm, structural differentiation starts; wall ingrowths can be seen in those cells that abut onto the integumentary tapetum (Figs. 6 and 7) and are absent from the wall lining enclosing the liquid endosperm.

3.2.3. Heart Stage

The appearance of the cotyledon primordia give the embryo a heart shape. During early heart stage, all the cells in the embryo proper, including those of the cotyledon primordia, are densely stained (Fig. 8). The general staining intensity is much higher than that of the globular stage. Progressive vacuolation occurs in the cytoplasm of most of the cells in the embryo proper, especially those in the cotyledon primordia (Fig. 9); cell expansion follows. The embryo proper turns pale green, indicating the maturation of the chloroplasts. By late heart stage, the embryonic axis is clearly defined. The meristems of the shoot and root can be observed with a clearly delimited procambial region (Fig. 10).

In the suspensor, cell divisions continue at the terminal region into early heart stage, then cease (BRADY 1972). The total number of cells by actual count is 200 ± 25 (BRADY 1972). The basal suspensor cells are highly vacuolated and are larger than the rest of the suspensor cells (Fig. 8). A basal-terminal gradation in nuclear size exists, with the largest nuclei in the basal suspensor cells (Fig. 8). The general staining intensity of the suspensor remains more or less constant through heart stage except for the basal suspensor cells which show a slight decrease. Wall ingrowths are best developed at this stage, especially in those cells which abut directly on the integumentary tapetum (Figs. 8 and 11). Furthermore, these cells remain highly cytoplasmic with numerous organelles (Fig. 11). The overall size of the suspensor is still increasing.

In the cellular endosperm, continued mitotic activity near the expanding cotyledons allows the cellular endospermic sheath to accommodate the expanding embryo (Fig. 12). This mitotic activity enables the cellular endosperm to separate the embryo from the liquid endosperm. Cytologically, the heart stage and globular stage cellular endosperm are similar. The space observed between the embryo and the cellular endosperm in some prepara-

tions (Fig. 9) is most likely a fixation artifact since there is no apparent digestion of the cellular endosperm next to the embryo proper. This observation will be studied further with the scanning electron microscope.

3.2.4. Cotyledon and Maturation Stage

Accelerated cell expansion in the cotyledon primordia indicates the start of the cotyledon stage which proceeds with a gradual filling of the endosperm cavity by the cotyledons. During the early cotyledon stage, the cells in the suspensor show cytological characteristics similar to those in the heart stage, except for a decrease in the overall staining intensity. The suspensor cells at the terminal neck region have enlarged slightly and the suspensor is clearly distinct from the embryo proper (Figs. 13 and 14). It is at this stage that the suspensor attains its maximum size: approximately 800 μm in length and 450 μm wide at the base. The total number of cells remains at approximately 200 (BRADY 1972). The wall ingrowths which have formed in the suspensor cells adjoining the embryo proper are not as well developed as those lower down (Fig. 14).

In the embryonic axis, differentiation continues. At mid-cotyledon stage, starch grains as determined by the PAS stain can be found in the embryonic axis (Fig. 15), whereas very few or none can be found in the expanding cotyledons (Fig. 16). By late cotyledon stage, the procambium is fully differentiated, the shoot and root meristems are well organized, and a pair of leaf primordia have emerged (Fig. 17). Vascular tissues start to differentiate within the cotyledons at this time. The major storage bodies are formed when the endosperm cavity has been completely filled by the cotyledons. At maturity, the cotyledons and embryonic axis are filled with starch grains and protein bodies as determined by positive PAS and aniline blue black staining respectively (Figs. 18 and 19).

In the maturation stage, marked changes occur in the suspensor. Additional wall material is deposited in the walls of the suspensor cells, and most of the wall ingrowths become less pronounced (Figs. 20 and 21); in most of the suspensor cells, the vacuoles are no longer present and what might be storage substances are observed. Lipid droplets and starch grains can be found in the cytoplasm of these cells (Fig. 20; ultrastructural observations, YEUNG, unpublished). The majority of the suspensor cells persist through maturation. A few remain vacuolated with fewer storage bodies (Fig. 21). The cytoplasm of these cells eventually breaks down into amorphous material.

The endosperm shows extensive changes through early cotyledon stage to complete maturation. During early cotyledon stage, active cell division of the cellular endosperm can be observed at the corners of the expanding cotyledons. At the same time, wall ingrowths start to appear in the wall lining enclosing the liquid endosperm (Fig. 22). The wall ingrowths first appear on

the side nearest the funiculus and soon spread through the remaining area. The formation of these ingrowths progresses towards the chalazal region of the seed. The volume of the liquid endosperm increases about six-fold from the heart stage to mid-cotyledon stage (Fig. 1). At mid-cotyledon stage, the expansion of the cotyledon is extremely fast and the cellular endospermic sheath stretches and finally breaks at maturation stage. However, the cellular endosperm surrounding the embryonic axis and near the micropylar end of the seed remains intact through maturation. Storage substances similar to those in the suspensor can be found in the cytoplasm of these cellular endosperm cells (Fig. 23). In addition, some cellulosic substance as demonstrated by the PAS stain is secreted by these cells (Fig. 23). These cells remain in a similar state through the maturation of the seed.

3.3. Maternal Tissues

The development of the vascular tissue and the morphological changes in the epidermis of the inner integument (integumentary tapetum) deserve particular attention since nutrient may be transported from the plant to the developing seed and embryo via these two tissues.

3.3.1. Vascular Tissues

Only one vascular trace enters each seed. As this trace enters the funiculus region, two recurrent vascular traces branch off, terminating in the integument towards the micropylar end. The pre-chalazal vascular trace terminates at the chalazal end; there is no post-chalazal vascular bundle. Although the maturation of the vascular elements is faster in the pre-chalazal trace, mature vascular tissues can be found in the two recurrent vascular traces at early globular stage (Fig. 24). As the seed matures, further differentiation occurs in the procambium within the seed coat, resulting in a network of vascular tissues. These vascular tissues are very well developed at the cotyledon stage.

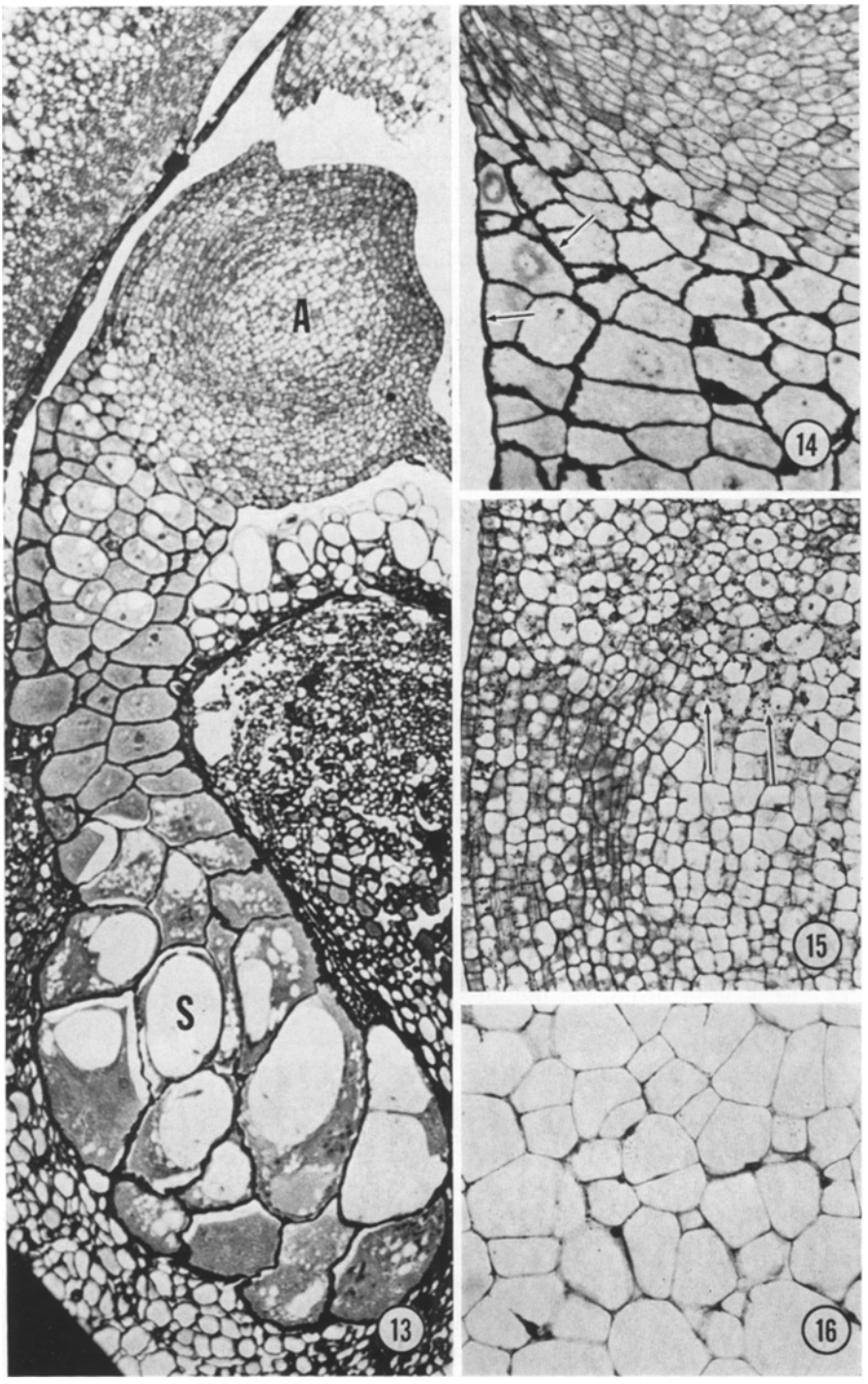
Plate 4. Figs. 13–16. The embryo at early cotyledon stage.

Fig. 13. Median longitudinal section of the embryonic axis (*A*) and the suspensor (*S*). The suspensor has attained its maximum size at this stage. The basal suspensor cells are highly vacuolated. ×150

Fig. 14. Longitudinal section of the embryo proper/suspensor junction. Wall ingrowths (arrows) are not as well developed in these suspensor cells as in those at the base of the suspensor; cells in the upper region also remain highly cytoplasmic with few vacuoles. ×420

Fig. 15. Longitudinal section of the embryonic axis showing the presence of starch grains (arrows). ×420

Fig. 16. Longitudinal section of the developing cotyledon showing no deposition of starch grains at this stage. ×500



Figs. 13–16

3.3.2. Integumentary Tapetum

Active cell division occurs in the tapetal cells during early seed development. Two layers of tapetal cells are often found near the micropylar end of the embryo sac (Figs. 2 and 4). Prior to the late heart stage, most of the tapetal cells have a dense cytoplasm; the nucleus and the nucleolus are prominent (Fig. 25). As the embryo approaches cotyledon stage, rapid expansion occurs in the seed. This causes all of the tapetal cells, except those near the micropylar end, to become elongated such that their long axis runs parallel to the axis of the embryo sac (Fig. 26). However, they appear to remain intact at the cotyledon stage of embryo development.

4. Discussion

Classical studies of the embryogeny of leguminous species (BROWN 1917, WEINSTEIN 1926, COOPER 1938) for the most part did not consider the interrelationship between different organs and tissues of the developing seed. In the present study, structural ontogeny is interpreted as part of a dynamic system and the possible functional significance of different organs and tissues will be discussed.

The most striking observations from this study are, 1. the rapid development of the suspensor, 2. the development of the cellular endosperm, especially the timing of the formation of wall ingrowths, and 3. the separation of the embryo from the liquid endosperm by the endospermic sheath.

The formation of wall ingrowths can be observed in the suspensor as early as the proembryo stage. The staining intensity and the cytological characteristics of these cells as shown in Fig. 11 suggest that they maintain a high degree of activity up until the heart stage. These cytological features agree with previously published reports on the biochemical aspects of embryo development in this species. During early embryogeny, the protein content of the suspensor is slightly higher than the embryo proper and there is a gradual accumulation of protein in the suspensor through development (WALBOT *et al.* 1972 a, SUSSEX *et al.* 1973). Furthermore, there is an increase in template

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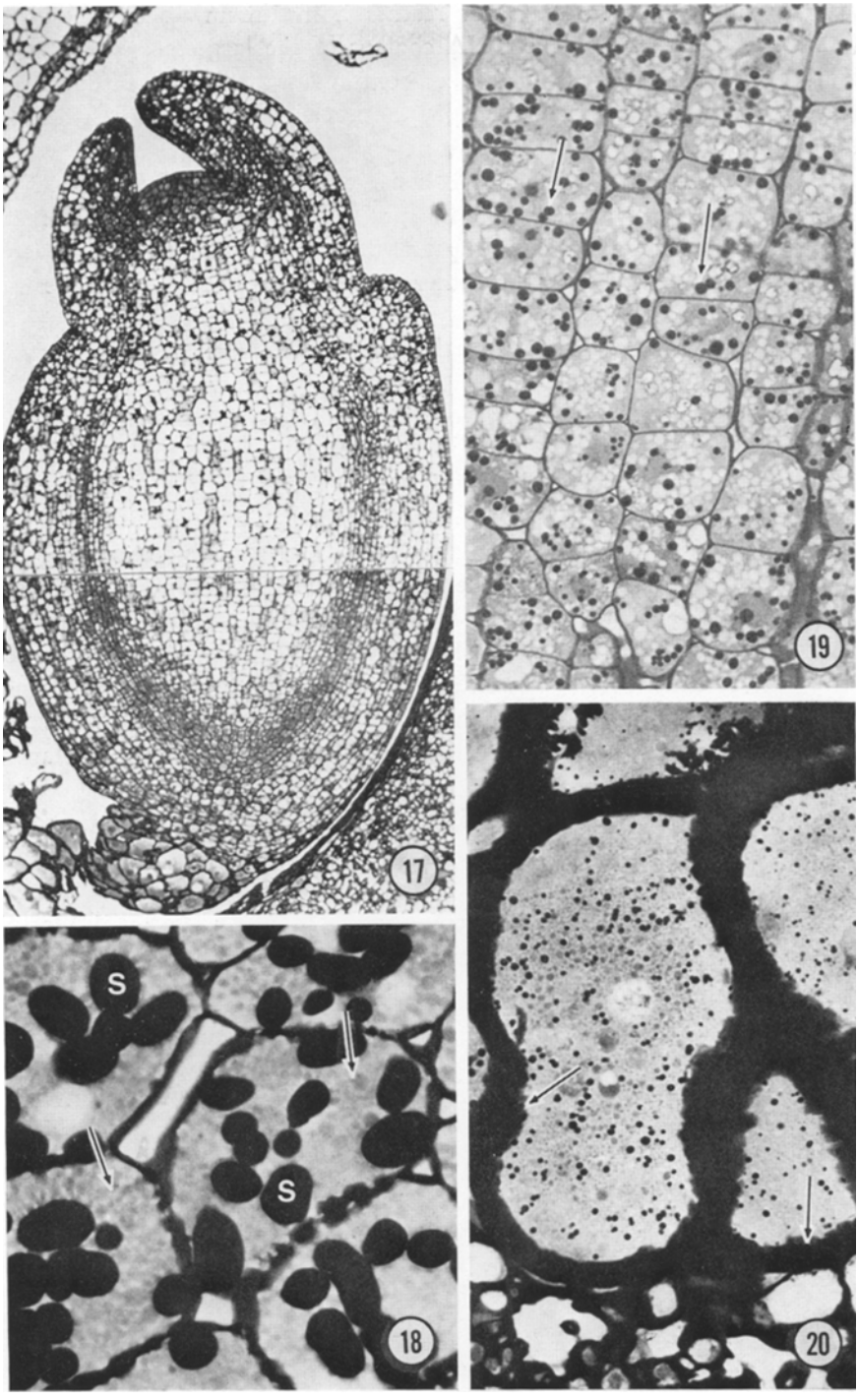
Plate 5. Figs. 17–20. Embryo at the late cotyledon, early maturation stages.

Fig. 17. Median longitudinal section of the embryonic axis at late cotyledon stage showing well organized apical meristems of the shoot and the root and a well differentiated procambium. A pair of leaf primordia have also formed. ×180

Fig. 18. Cotyledonary cells at maturation stage showing numerous starch grains (*S*) and protein bodies (arrows). ×800

Fig. 19. Embryonic axis at maturation stage showing numerous starch grains (arrows) and small vacuoles in the cytoplasm of the cells. ×1,000

Fig. 20. Suspensor cells at maturation stage. Additional wall material has been deposited in the walls of the suspensor cells and the ingrowths are less pronounced (arrows). Storage substances can be detected in the cytoplasm of the cells. ×750



Figs. 17–20

activity in the suspensor, which reaches a maximum at late heart stage (CLUTTER *et al.* 1974). The DNA content of the largest suspensor cell can reach 8192 C, DNA being synthesized continuously through seed development (BRADY 1973). As shown in Fig. 8, the nuclei in the basal suspensor cells are much larger than those at the neck region. This agrees well with the quantitative determination of DNA content in these cells (BRADY 1973).

The appearance of starch grains and lipid bodies in the suspensor at maturation stage coincides with their appearance in the cotyledons. However, the physiological significance and whether they serve as storage substances in the suspensor is not known at present.

Does the suspensor play a role in the early development of the embryo? It has been shown in *Eruca* (CORSI 1972) that the growth of the early-heart embryo is greatly improved by the presence of the suspensor. Recent results from *in vitro* culture experiments with *P. coccineus* also indicate that there is a requirement for the presence of the suspensor prior to late heart stage (NAGL 1974, CIONINI *et al.* 1976). It is generally known that progressively younger embryos require more complex nutrients for their growth (STEEVES and SUSSEX 1972). A culture medium similar to that of the endospermic fluid cannot support the growth of the globular stage embryo of *P. vulgaris* (SMITH 1971). Therefore, this obligate requirement for the suspensor in *in vitro* culture experiments, as well as the suspensor's early differentiation and high rate of macromolecular synthesis *in vivo* (SUSSEX *et al.* 1973, CLUTTER *et al.* 1974) suggest strongly that the suspensor is playing an important role during early embryogeny.

The presence of wall ingrowths in the suspensor of this and other species (PATE and GUNNING 1972, GUNNING and PATE 1974) further strengthens the above hypothesis. This type of differentiation increases the surface area available for solute transport or exchange and for this reason wall ingrowths are thought to be a feature of cells which are specialized to perform this

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Plate 6

Fig. 21. Degenerating suspensor cell showing extensive cytoplasmic vacuolation. ×800

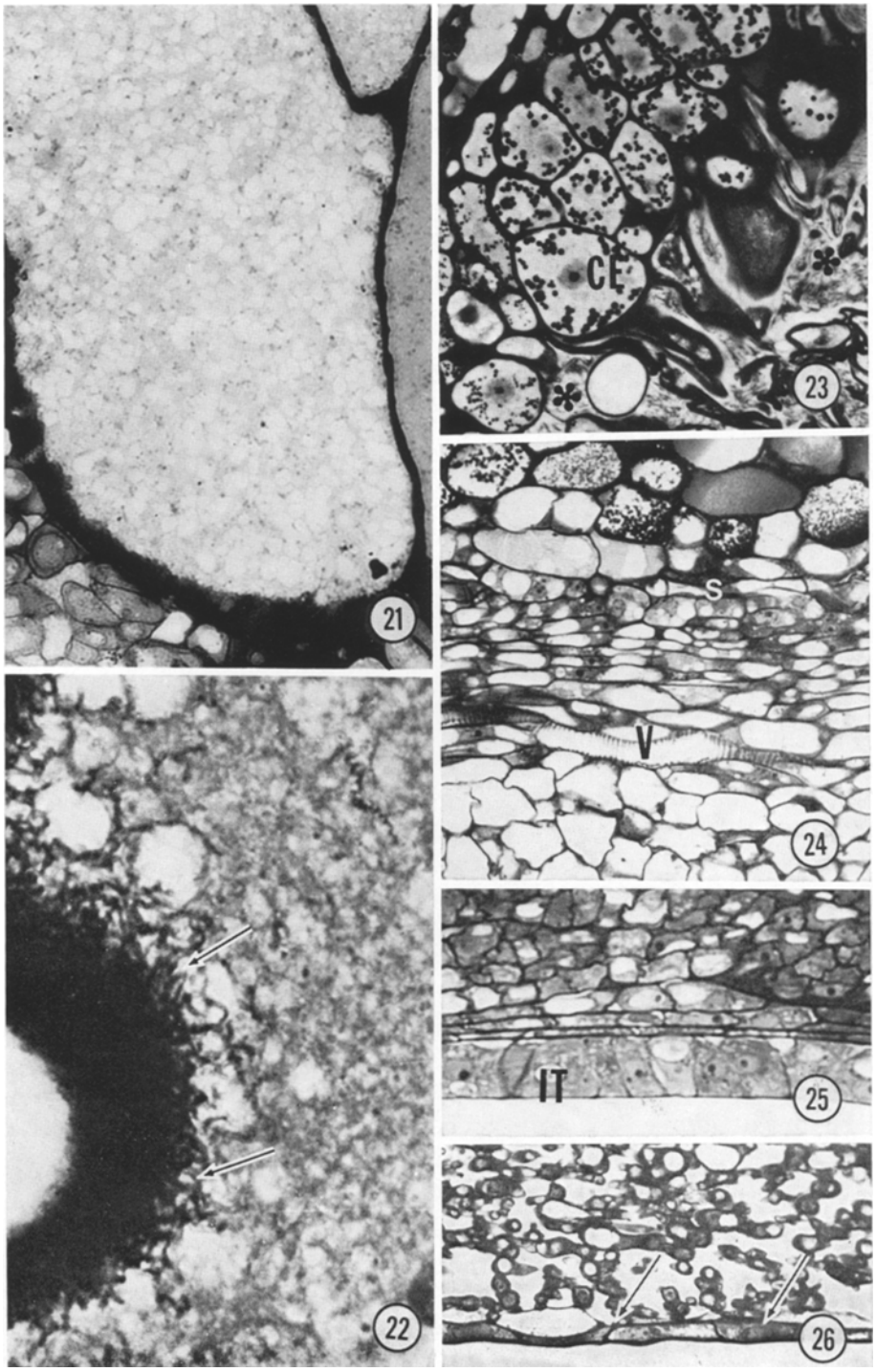
Fig. 22. Wall ingrowths (arrows) in the embryo sac lining of the liquid endosperm at the cotyledon stage. ×1,100

Fig. 23. The formation of storage bodies in the cytoplasm of the cellular endosperm (*CE*). In addition, cellulosic substance (*) is also secreted by these cells at maturation stage. ×450

Fig. 24. Mature vascular tissues, *i.e.*, sieve tube members (*S*) and vessel elements (*V*), in the recurrent vascular traces of the seed coat at early globular stage of embryo development. ×650

Fig. 25. Integumentary tapetum (*IT*) at an early stage of embryo development. Note the dense cytoplasm and prominent nucleoli of the cells in this tissue. ×1,000

Fig. 26. Integumentary tapetum (arrow) at cotyledon stage. The cells are highly elongated but appear to be intact. ×500



Figs. 21–26

function. In fact, there is a strong correlation between the appearance of wall ingrowths and the onset of solute flow (GUNNING *et al.* 1968, PATE *et al.* 1970). If this is the case in this species, the appearance of wall ingrowths at the proembryo stage may indicate that the suspensor can function very early on in solute transport. Since the suspensor protrudes well into the seed coat and wall ingrowths are extremely well developed in the neck suspensor cells that abut onto the integumentary tapetum, direct absorption and transfer of nutrients from the maternal tissues to the developing embryo via the suspensor is possible, by-passing what has been thought to be the "conventional" route —from the endosperm to the embryo. In *Phaseolus*, the micropylar region is well supplied with vascular traces, and mature vascular tissues have been observed in the two recurrent vascular traces early in embryogeny.

The vacuolation pattern of the suspensor may reflect a shift in activity from the basal region to the neck region. In actively secreting cells of the septal nectary glands of *Gasteria trigona,* vacuoles are extremely reduced in size; dense cytoplasm with numerous mitochondria and wall ingrowths can be observed in these cells. But in the post-secretory stage, a large central vacuole is formed and the cytoplasm is restricted to a thin peripheral layer (LÜTTGE and SCHNEPF 1976). Similar changes have also been reported in the digestive gland of the Venus flytrap (SCHWAB *et al.* 1969). Therefore, the observed changes in the vacuolation pattern of the suspensor suggest a gradual shift of activity from the basal suspensor cells to the neck suspensor cells as development of the embryo progresses from proembryo to late heart stage.

Cellular endosperm forms very early in embryogeny and is extremely well developed by heart stage, especially around the neck region of the suspensor. Structural differentiation also occurs very early in these endosperm cells, wall ingrowths are present in those cells that abut onto the integumentary tapetum. Since nutrients from the maternal tissues may reach the embryo through these tapetal cells, the presence of wall ingrowths in the cellular endosperm cells along the tapetal cells could facilitate the transfer of nutrient from the maternal tissues to the developing embryo. At early cotyledon stage, the appearance of ingrowths in the wall-lining enclosing the liquid endosperm coincides with the rapid increase in the volume of endospermic fluid. The appearance of wall ingrowths in the embryo sac suggests that the entire surface of the embryo sac can take part in the uptake of nutrient from the maternal tissue even though the ingrowths are not equally extensive over the entire surface of the embryo sac. In this regard, the formation of these ingrowths is similar to that of pea (MARINOS 1970).

The close association of the cellular endosperm and the neck suspensor cells may be of functional significance. Cytologically, these cells appear to be metabolically active; wall ingrowths can be found in both types of cells in this region. Like the suspensor at maturation stage, the cellular endosperm is filled with starch grains and lipid bodies. Therefore, they may be a func-

tional complex, cooperating to supply the necessary requirements for the growth of the embryo especially during early development, *i.e.*, prior to the cotyledon stage. Even after the cotyledons have filled up the endosperm cavity, the cellular endosperm-suspensor complex may still be able to function as the major site of entry for nutrients. As noted earlier, starch grains start to accumulate in the embryonic axis before they can be observed in the expanding cotyledon. This observation could indicate the existence of gradients of solute concentration within the embryo with the highest at the embryonic axis near the endosperm-suspensor junction. This observation seems to support the argument that the endosperm and the suspensor may be able to regulate the flow of solute into the embryo (NEWCOMB 1973).

The classical interpretation has considered the embryo to be directly embedded in the liquid endosperm, which serves as the important source of nutrients for the developing embryo (MAHESHWARI 1950). In *Phaseolus* and some other leguminous plants, in fact, the embryo is physically separated from the liquid endosperm by the endospermic sheath (STERLING 1955, MARINOS 1970). Since the liquid endosperm is absorbed, there is little doubt that the endosperm can serve as a nutrient reservoir for the growing seed. But whether the endosperm serves as the sole nutrient resource during early embryogeny is still open to question. The endosperm in *P. coccineus* is still developing at heart stage and has not yet reached its maximum volume (Fig. 1). Therefore, the contribution of the liquid endosperm to the growth of the early embryo may not be very significant. Similar questions have been put forward by researchers studying the embryogeny of *Capsella* (SCHULZ and JENSEN 1969) and *Helianthus* (NEWCOMB 1973).

The liquid endosperm in *Phaseolus* and other leguminous plants contains different classes of hormones including auxin, cytokinins and gibberellins (SMITH 1971, EEUWENS and SCHWABE 1975) and in *Echinocystis lobata*, the liquid endosperm also has the ability to metabolize abscisic acid (GILLARD and WALTON 1976). Growth substances have also been found in the suspensor (ALPI *et al.* 1975). Therefore, besides serving as a temporary nutrient source, the endosperm, together with the suspensor, may play a morphogenetic role in the regulation of the growth of the embryo. It is also known that liquid endosperm has a high osmotic pressure. In *Phaseolus vulgaris*, the osmotic pressure is 0.7 osmolar at heart stage and 0.5 osmolar at the late cotyledon stage (SMITH 1971, 1973). This high osmotic environment may be essential for the normal development of the embryo, and may also prevent it from precocious germination (NORSTOG 1967, 1972). But the effect of this high osmotic environment on nutrient uptake by the embryo is unknown. The cellular endospermic sheath may play an important role in regulating nutrient flow within the seed, with the wall ingrowths providing a mechanism for the uptake of nutrients at a relatively high osmotic pressure.

In summary, this anatomical study has shown a close interplay between

various tissues and organs of the developing seed. It is suggested that the suspensor and the endosperm play an important role in nutrient regulation during early embryogeny. Establishing more definitive roles for the suspensor and the endosperm will require fine quantitative analyses of hormone and nutrient movements during embryo development. However, the evidence at hand certainly indicates that the suspensor is more than an attachment tissue between the embryo proper and the maternal tissues.

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