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ONTOGENY AND CYTOCHEMISTRY OF THE CHALAZAL PROLIFERATING CELLS OF *CAPSELLA BURSA-PASTORIS* (L.) MEDIC.

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SUMMARY

This paper describes the ontogenic development and cytochemistry of a group of cells which proliferate from the chalaza of the ovule of *Capsella bursa-pastoris*. These cells arise from nucellar tissue in the chalaza at the time of embryo development and develop a consistent morphology which seems to protrude into the embryo sac. Azure B staining and intensity of incorporation of nucleic acid precursors indicates a high rate of activity of these cells up to the time that the embryo is in the heart stage. They then begin to disintegrate quickly with but a few cells remaining in the mature seed. The evidence suggests that they may play a physiological role in early embryo development.

INTRODUCTION

In the course of a study on higher plant embryogenesis (Pollock and Jensen, 1964), attention was called to a group of cells which proliferate in the chalaza of the ovule of *Capsella bursa-pastoris* (L.) Medic. The growth of these cell parallels that of the embryo for a time but they then become hypertrophied and disintegrate well before the embryo is fully mature. They do not differentiate into any distinct morphological structure or in any way resemble an embryo. Cellular proliferations arising from haploid cells of the embryo sac or from diploid nucellar cells to become an embryo characterize many species of plants (Maheshwari, 1950). In *Capsella*, however, the consistent development and regular pattern of growth of the chalazal proliferating cells and their regular abortive pattern does not immediately suggest polyembryony or any form of apomixis. This paper is about the ontogeny of these chalazal proliferating cells of *Capsella* and describes some aspects of their nucleic acid metabolism.

MATERIALS AND METHODS

Histology

Siliculae of various sizes along the developing inflorescence of *Capsella* were collected and fixed in Navashin's fluid (Jensen, 1962), FPA (formalin, proprionic acid and ethyl alcohol) and Carnoy's fluid (Johansen, 1940). The lateral portions of the siliculae were removed to allow for better penetration of the fixatives. All chemically fixed material was embedded in paraffin by the standard *t*-butyl alcohol series. The tissues were sectioned at 5–10 μ and stained with Heidenhain's iron haematoxylin; counter-stained with Fast Green. These materials were used for a histological study of the chalaza of the developing ovule from the pre-fertilization period up to the mature seed.

Incorporation studies

After cutting open the siliculae, terminal inflorescences of *Capsella* were submerged in solutions of concentrations of [^{3}H]thymidine and [^{3}H]uridine (5 μCi/ml) for 24-hour periods. The inflorescences were not excised from the plants during this 'dip' method of treatment. Following exposure to the labelled precursors the siliculae were collected from the plant, freeze-substituted and paraffin embedded (Jensen, 1952). These materials were sectioned at 5 μ thickness and liquid emulsion autoradiographs prepared using Ilford G-5 emulsion. The slides were placed in the dark for 5–14 days, developed, then stained with Azure B.

RESULTS

Ontogeny

Plate 17, Nos. 1–6 show the progressive development of the cells which proliferate in the chalaza of *Capsella*. At the time of fertilization the antipodals are still in evidence in the embryo sac and there is little indication of any cytomorphological changes taking place in the nucellus of the chalaza (No. 1). Soon after fertilization, however, cells of the nucellus in the chalaza divide anticlinically with respect to the curvature of the embryo sac to begin the formation of a volcano-shaped mound. The antipodals at this time can be observed to disintegrate in the distal pocket formed by the proliferation (No. 2). These first-formed proliferating cells of the nucellus stain rather densely and are closely packed together (No. 3). By the time the embryo has reached a full globular stage these chalazal cells have proliferated excessively through producing several tiers. The median portion of the mound is characterized by cells which are large and elongated parallel to the long axis of the mound. These cells were the first-formed and are now the oldest of the group (No. 4). The distal end of this tissue mound is also characterized by some cells which have died and are now in the process of disintegration leaving only closely appressed, deep staining cell wall material (Nos. 4, 5 and 6). Evidently, cellular disintegration begins early starting with the first-formed cells of this proliferation, but at this time the rate of disintegration is much slower than the rate of cellular proliferation. All of the cells which, at one time or another characterize the mound, have an enlarged nucleus which stains deeply for DNA and a dense cytoplasm which stains deeply for RNA as shown by haematoxylin/fast green staining and this distinguishes them clearly from the other ovular tissue in the chalaza. Growth appears to be outward from the nucellus toward the embryo sac, with most of the divisions occurring at the base of the mound in the nucellus.

The breakdown of the chalazal proliferating cells is somewhat accelerated at about the time the embryo is in the late 'heart' to early 'torpedo' stage, as shown by dense cytoplasmic staining and cellular disorganization (Plate 18, No. 7). Although the more distal cells of the mound are first affected, it is not uncommon to see elongated and more proximally located cells disintegrating at this time. Cell proliferation has slowed considerably at this stage and effectively stops. It is at this transitional step between the cessation of cell division and the acceleration of cellular disintegration that some cell differentiation is apparent at the base of the chalazal tissue mass (No. 8). It is hard to say whether this newly-formed tissue layer is derived from part of the proliferating mound or is from the nucellus. In any case, a continuous sheet of cells with very thick walls differentiates which completely envelops the embryo sac and is immediately adjacent to it. This newly formed structure is only one cell-layer thick around the embryo sac

but it is from three to four cells deep in the chalaza which contain densely staining, resinous-like substances (No. 8). Although the histogenesis of ovular integuments is sometimes complex and confused, this layer may be referred to as a perisac in the younger ovule, which probably becomes part of the inner coat of the mature seed. Finally, a progressive wave of disintegration moves proximally through the mound midway through ovule development and is essentially complete with only a few small cells and cell remnants in evidence by the time the embryo is fully developed in the mature seed (Nos. 7–11).

Incorporation studies

Using the 'dip' method for treating *Capsella* inflorescences with labelled precursors, none of the tritiated nucleosides appear to be incorporated into the very young embryo (globular stage) or into the endosperm, while the incorporation appears to be very good in the chalazal proliferating cells and in the other tissues of the ovule (Plate 19, Nos. 12 and 13). This difference is perhaps best explained on the basis of differential cell permeability for, with the addition of 0.25 M mannitol to the medium, the osmotic property of the cells is altered and the young embryo and endosperm incorporate labelled precursors very well. Without the addition of mannitol there is evidence of slight incorporation into the endosperm and embryo during later stages of maturity, i.e. beginning with the 'heart' stage (No. 14). [^{3}H]Thymidine is taken up quite heavily by the chalazal proliferating cells from the very moment the proliferating begins. Subsequent growth is rapid and is characterized by a high rate of incorporation of the precursor by the time the embryo is four-celled (No. 13). At this time, there appears to be a proximal-to-distal gradient of incorporation intensity in the chalazal cell mass and for at least two or three cells deep. Later on, midway through embryo growth, active incorporation of [^{3}H]thymidine appears to be restricted to the more basal or proximal of the chalazal proliferating cells even though the distal cells of the proliferation are disintegrating (No. 15).

[^{3}H]Uridine incorporation into these cells appears to be quite uniform during all stages of their growth. The relative amount taken up by these cells, however, greatly exceeds that in any other cells in the ovule during the first half of embryogenesis, i.e. up to the time when they mostly cease proliferating uniformly and begin to break down (No. 16). A drop in uridine incorporation parallels the decline in cell proliferation and subsequent cellular disintegration.

Discussion

The special group of cells which are readily seen in the chalaza of sections of *Capsella* ovules has never been identified in terms of their origin or fate. That they clearly arise from nucellar tissue and protrude more-or-less into the embryo sac suggests that they should be considered in light of one of two hypotheses. The first is that these cells represent an aberrant structure which has neither an adaptive nor a deterrent value in the life of *Capsella*. More specifically, they may represent an abortive form of adventive embryony since they are characterized in the same manner of origin and staining properties in the early stages of growth as are true adventive embryos reported in other plant groups (Maheshwari, 1950). The early morphogenesis of the chalazal proliferating cells does not in any way resemble that of the early embryo, however. Further, the appearance of these proliferating cells is regular and consistent in every ovule of *Capsella* and is not the random feature which usually characterizes most forms of apomixis. In this

respect the idea that these cells which arise from nucellar tissue represent an adventive embryo which aborts regularly does not seem plausible.

The second hypothesis suggests that these cells act as a metabolic strainer for nucleic acid precursor materials going into the embryo during its later phases of growth. This suggestion gains support from the following observations.

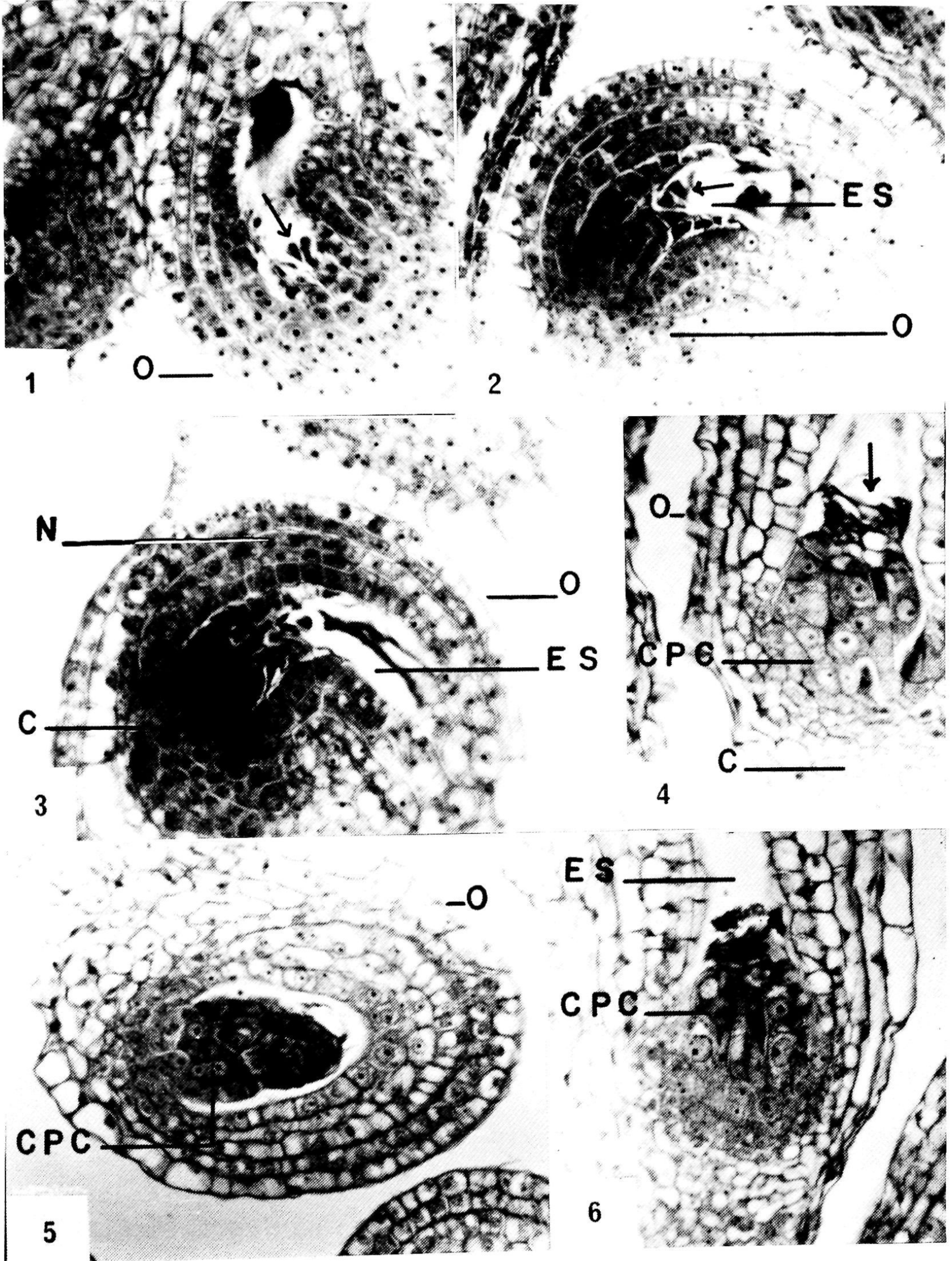
(1) Using the 'dip' method for exposing intact ovules to solutions of tritiated thymidine and uridine the label is incorporated heavily by cells in all of the parts of the ovule except in those cells within the embryo sac. Since this inability of the cells within the embryo sac to incorporate exogenous precursors is apparently accounted for on the basis of their permeability, it would appear that endogenous nucleosides would have comparable difficulty moving in and out of the structures within the embryo sac. This would be true, however, only for young embryos. Since beyond the 'heart' stage embryos can incorporate exogenous precursors one could assume that endogenous precursor movement would be more common in later stages of growth. It is at this time that the chalazal proliferating cells could provide a ready supply of such materials to the growing embryo in intact ovules.

(2) The vascular strands coming through the funiculus proliferate and terminate in the chalazal region of the ovule and in close proximity to the cellular proliferation in this area. Nutrient materials coming through the funiculus would be deposited firstly in the chalazal region.

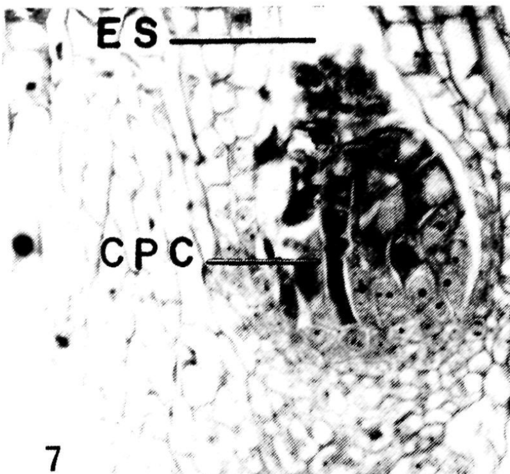
(3) The chalazal proliferating cells incorporate labelled nucleic acid precursors more heavily than any other group of cells within the ovule. And the precursors appear to be concentrated in these cells up to the time of their disintegration; a time when the embryo is rapidly maturing and when most reserves in the ovule may be depleted. What happens to the nucleic acid materials concentrated in these cells after disintegration is unknown. But it is reasonable to suggest that they are used by the growing embryo.

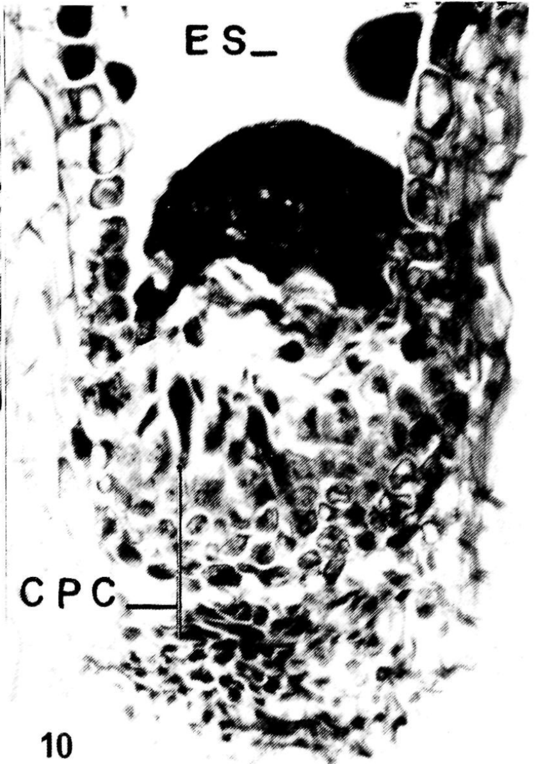
These observations lend reasonable support to the idea that the chalazal proliferation plays a significant role in embryo growth. The embryo during early growth seems to gather precursor molecules from reserves in the embryo sac or from breakdown. During the same period of growth the chalazal proliferating cells are very active in terms of nucleic acid precursor incorporation and probably nucleic acid synthesis. Still, this activity is not a prelude to a recognizable well-developed structure for later, as the embryo is maturing through the torpedo stage, the chalazal proliferation breaks down, freeing its nucleic acid products. Seemingly, these would be available to the embryo which could possibly incorporate them.

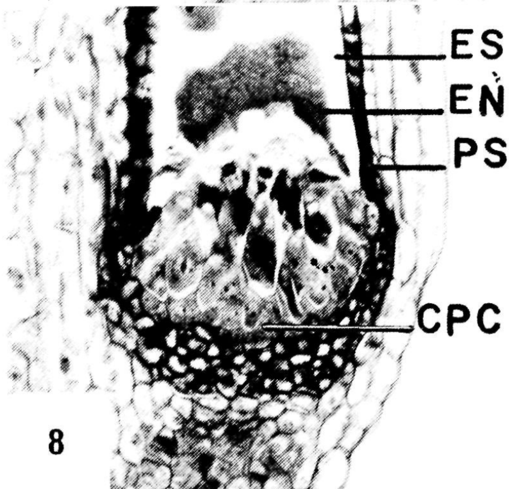
It would not be unreasonable to conclude that there exists a very intimate relationship between the chalazal proliferating cells and the embryo. The regular appearance of these cells in every ovule and their regular pattern of behaviour would suggest a significant adaptive relationship. This is not to suggest that embryo growth is absolutely dependent on these cells. It is possible that such growth could proceed normally should the chalazal proliferating cells be removed and their contents not made available to the embryo at the time of their disintegration. In this case other sources of precursors would very well be at hand since the chalazal cell contents would merely add to the pool of available molecules. The alternative would be to imply a direct dependency of the embryo during its later growth phase upon the materials provided by the chalazal proliferating cells. It remains to test which of these alternatives represents the function of this unique group of cells in the chalaza of *Capsella*.

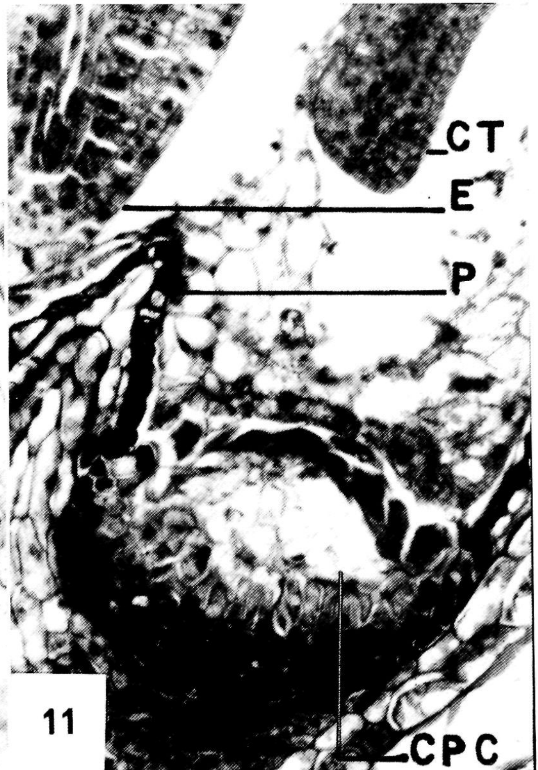


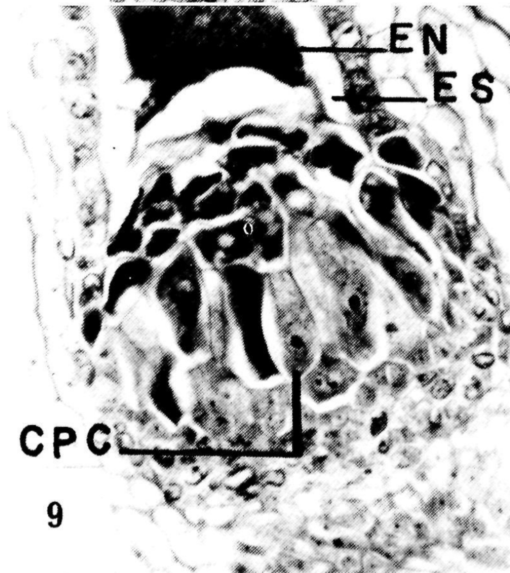
E. G. POLLOCK AND W. A. JENSEN— *CHALAZAL CELLS OF* CAPSELLA (*facing page* 416)











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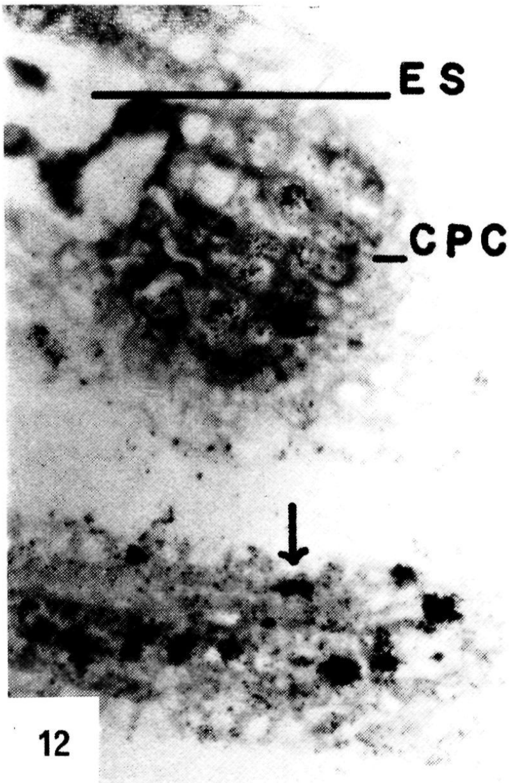

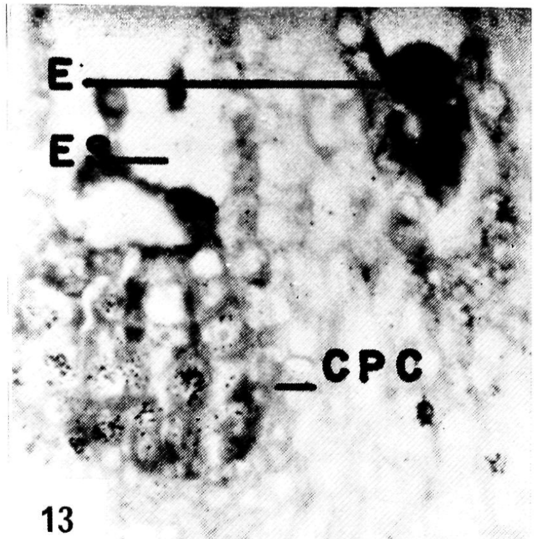

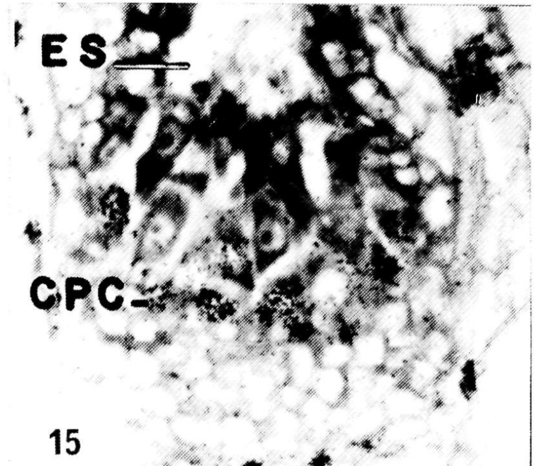

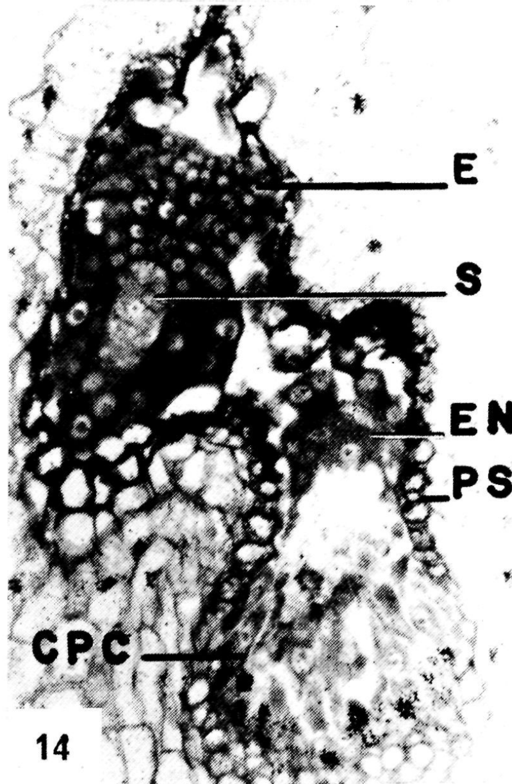

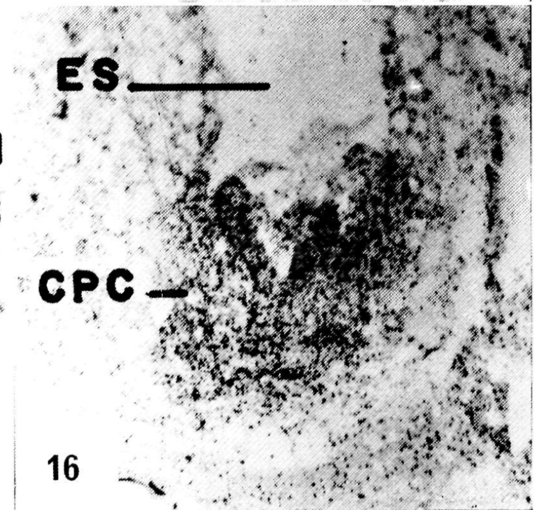

E. G. POLLOCK AND W. A. JENSEN—*CHALAZAL CELLS OF* CAPSELLA

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EXPLANATION OF PLATES 17–19

PLATE 17

Nos. 1–6. Sections through developing ovules of *Capsella* showing the progressive development of the chalazal proliferating cells. All sections stained with Heidenhain's Iron Haematoxylin and Fast Green. C, Chalaza; CPC, Chalazal proliferating cells; ES, embryo sac; N, nucellus; O, ovule.

No. 1. Young ovule sectioned through the chalaza revealing two of the antipodals (arrow) of the embryo sac. ×278.

No. 2. Disintegration of the antipodals (arrow) in the embryo sac. ×278.

No. 3. Beginning of cell proliferation in the chalaza. Note the dense staining reaction of these cells. ×278.

No. 4. The chalazal proliferation showing dark-staining, cell wall remains at the distal end due to early disintegration of the first-formed cells (arrow). ×533.

No. 5. An oblique section through the chalaza of the ovule showing a well-formed proliferation. ×278.

No. 6. The chalazal proliferation showing the large nuclei of the cells and some advanced terminal disintegration which has spread proximally to some of the medianly located cells. ×278.

PLATE 18

Nos. 7–11. Median longitudinal sections through the chalaza of *Capsella* showing the progressive growth and disintegration of the chalaza-proliferation concurrent with embryo maturation. All sections stained with Heidenhain's Iron Haematoxylin and Fast Green. CPC, Chalazal proliferating cells; CT, cotyledon; E, embryo; EN, endosperm; ES, embryo sac; PS, perisac layer.

No. 7. Cell elongation and breakdown as evidenced by the dark-stained cells in the chalazal proliferation. ×615.

No. 8. Differentiation of a well-structured cell layer around the embryo sac. In the chalaza it is about four cells deep. ×615.

No. 9. Almost complete distal disintegration of the chalazal-proliferation shown by the disappearance of characteristic cellular morphology and the increase in density. Note the large, elongate proximate cells. ×984.

No. 10. The chalaza of a near-mature ovule showing almost complete disintegration of the proliferation. ×984.

No. 11. Complete disintegration of the chalazal-proliferation in a mature seed. ×984.

PLATE 19

Nos. 12–16. Autoradiographs of [^{3}H]thymidine and [^{3}H]uridine incorporation into proliferating cells of the chalaza of *Capsella*. All preparations were stained with Azure B. CPC, Chalazal proliferating cells; E, embryo; EN, endosperm; ES, embryo sac; PS, perisac layer; S, suspensor of embryo.

No. 12. [^{3}H]Thymidine incorporation into the chalazal-proliferation and surrounding ovular tissue (arrow). ×615.

No. 13. [^{3}H]Thymidine incorporation into the CPC. Note the absence of silver grains over the four-celled embryo. ×615.

No. 14. [^{3}H]Thymidine incorporation into the embryo and endosperm compared with the chalazal-proliferation. ×615.

No. 15. [^{3}H]Thymidine incorporation into the proximal cells of the proliferation while disintegration characterizes the distal cells. ×984.

No. 16. [^{3}H]Uridine incorporation into the chalazal proliferating cells of the ovule. ×984.

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