BOT. GAZ. 139(1):81–86. 1978.

ABNORMALITIES IN AVOCADO (PERSEA AMERICANA MILL.) OVULE DEVELOPMENT[1]

E. TOMER AND M. GOTTREICH

Agricultural Research Organization, Volcani Center, Bet Dagan, Israel

Abnormalities in avocado (*Persea americana*) ovules at blossoming are explained by demonstrating their possible origin at some stage of ovule development. Degeneration of the embryo sac at the micropylar site may occur at the four-nucleate stage or at egg-apparatus organization, prior to or after the filiform apparatus has formed. The time of occurrence of degeneration decisively determines the appearance of the degenerate remnants. The absence of an embryo sac and the presence of a double embryo sac originate at the earliest stages of the nucellar primordium; a third abnormality, compartmentalization, is assumed to originate at the megaspore tetrad stage.

Introduction

TOMER, GOTTREICH, and GAZIT (1976) found that avocado ovules at blossoming included a large percentage of degenerate and abnormal ovules, many of which were difficult to identify or interpret due to either a blurred appearance or excessive irregularity. This study is concerned with the development of the avocado ovule and attempts to elucidate the origin and nature of previously encountered abnormalities. The normal and abnormal development of the avocado ovule was described by SCHROEDER (1940, 1952). Some new observations are recorded here.

Material and methods

Floral buds of *Persea americana* 'Fuerte,' 'Hass,' and 'Ettinger' at different developmental stages were fixed in FAA, dehydrated by TBA, Tissuemat-embedded, cut serially 10–15 μm thick, and stained with safranin-fast green. On February 22, 1973, developing inflorescences were sorted according to the stages present at that time; they provided the earlier stages. On March 7, 1975, buds from inflorescences starting to flower were sorted according to size; they provided the later stages. A total of 400 buds was examined.

Results

NORMAL OVULE DEVELOPMENT

Some phases of the normal development are reviewed to facilitate understanding of the abnormalities, and some new details are added. The timing of flower development, 6–8 wk before blossoming, agrees with SCHROEDER (1951). The ovule contains an embryo sac of the Polygonum type (SCHROEDER 1952). In the primordial nucellus a hypodermal archespore (fig. 1) becomes transformed into a megaspore mother cell (fig. 2) which by two successive meiotic divisions yields a tetrad of megaspores (SCHROEDER 1952) (fig. 3). Three megaspores degenerate (fig. 4) and the fourth, the chalazal one, continues to grow and becomes the mononucleate embryo sac (SCHROEDER 1952) (fig. 5). At the two-nucleate stage a distinct apex of the nucellus has developed, and in its cells there are three to five fast-green stained dots (fig. 6) that disappear before anthesis.

Cytoplasm begins to form around the nuclei of the four-nucleate embryo sac (fig. 6). At the eight-nucleate stage two assemblages of four nuclei are embedded in dense dark-staining cytoplasm at the poles of the embryo sac. Thereafter, the final organization of the egg apparatus and the central cell takes place. At anthesis the synergids have a conspicuous filiform apparatus (TOMER et al. 1976). We did not see formation of the filiform apparatus; this study included only the earlier stages of the egg-apparatus organization (fig. 7); hence we assume that this formation is one of the latest events to occur in embryo sac development.

ABNORMAL OVULE DEVELOPMENT

Abnormal development includes degenerated embryo sacs and deviations from the normal path of development that are not associated with disintegration.

DEGENERATION

Different types of avocado ovule degeneration at anthesis were described by TOMER et al. (1976) and classified according to the degree of deformation or irrecognizability of the degenerate remnants. In the majority of degenerate ovules a degenerate egg apparatus was seen, and the filiform apparatus proved to be the most stable part of it, still recognizable when all other parts were no longer identifiable. In a minority of degenerate ovules a small, formless mass without any resemblance to an egg apparatus or filiform apparatus was seen (TOMER et al. 1976).

The present study allows a division of the different forms of degeneration into two main groups based on their origin, using the presence or absence of the filiform apparatus as a criterion. Degeneration of the embryo sac first manifested itself clearly in the four-nucleate stage by affecting one of the nuclei pairs,

[1] Contribution no. 306-E, 1976 series, from the ARO, The Volcani Center, Bet Dagan, Israel.

Manuscript received January 1977; revised manuscript received May 1977.

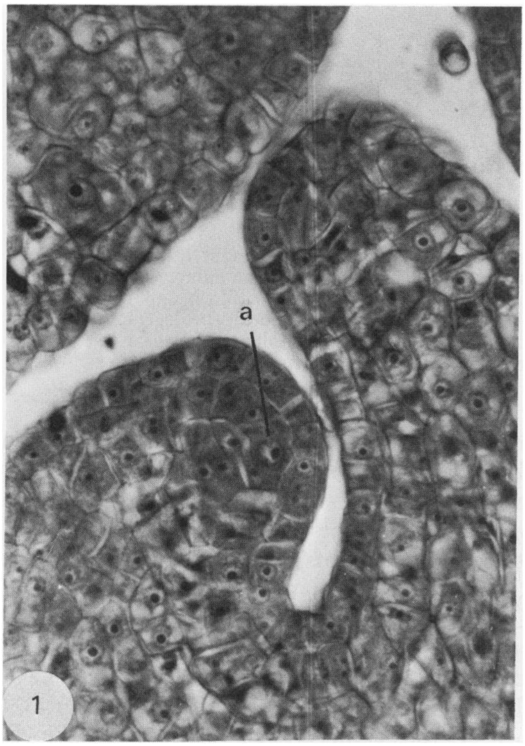

FIG. 1.—Hypodermal archespore (*a*); × 500

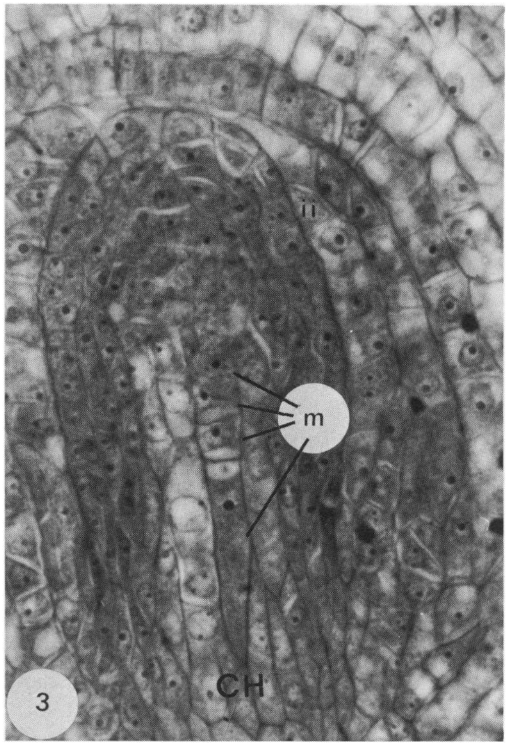

FIG. 3.—Tetrad of megaspores (*m*), the chalazal (*CH*) one as long as the remaining three together; inner integument (*ii*) near closing; × 550.

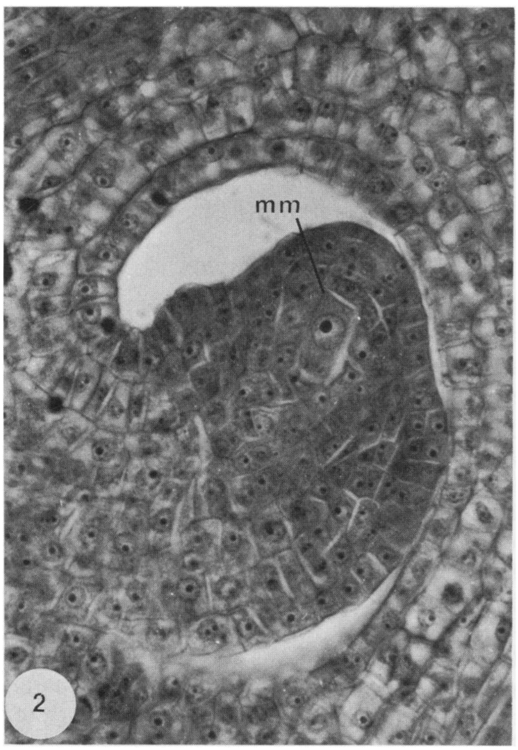

FIG. 2.—Megaspore mother cell (*mm*) in young nucellus with initiating integument; × 500.

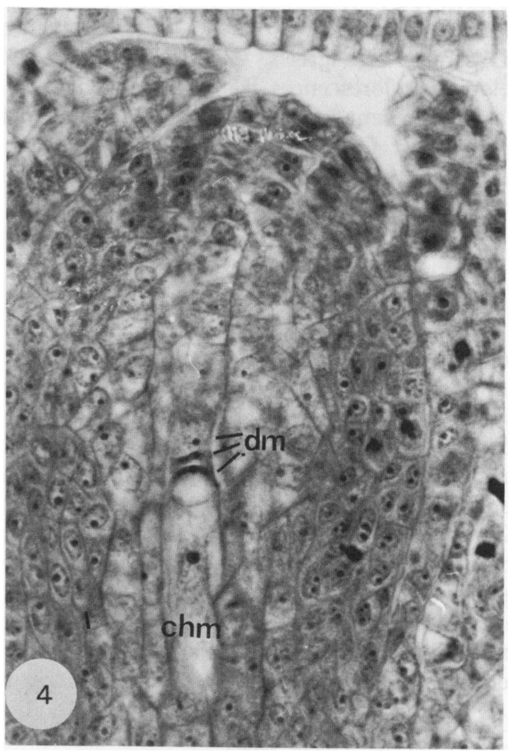

FIG. 4.—Three degenerate megaspores (*dm*) and the remaining chalazal (*chm*) one; inner integument not yet closed; × 550.

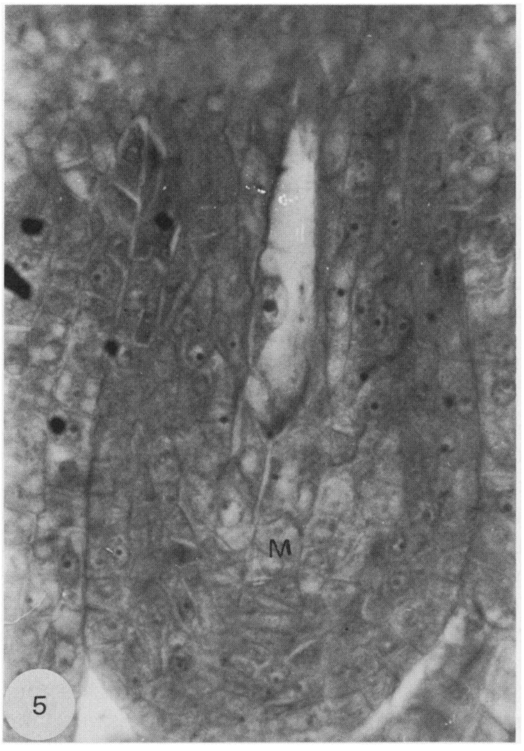

FIG. 5.—Mononucleate embryo sac; × 550. *M*, micropylar end.

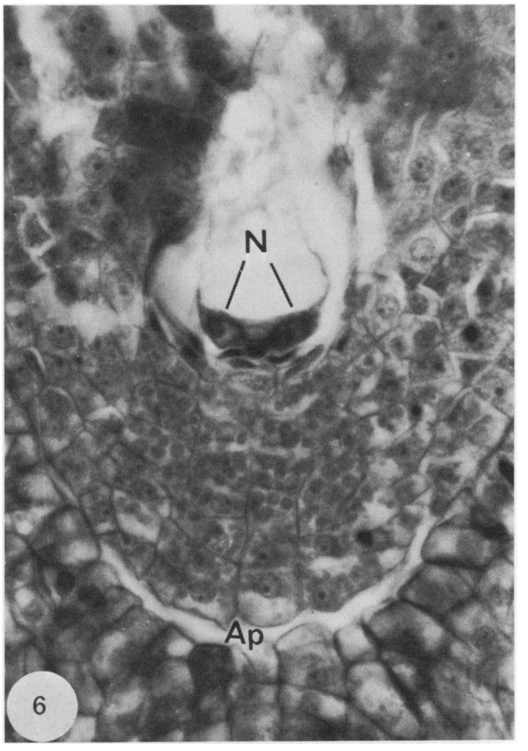

FIG. 6.—Nucellar apex (*Ap*) with dotted inclusions. Micropylar nuclei (*N*) of four-nucleate stage embedded in cytoplasm; × 550.

either the micropylar or the chalazal (fig. 8).[2] Degeneration of the micropylar nuclei at this stage leads to the smallest residues seen in the mature ovule at anthesis. A tiny, amorphous, opaque mass containing faint remnants of two nuclei may be all that can be detected. If degeneration occurs at an early stage of egg apparatus organization, prior to the formation of a filiform apparatus, the resulting degenerate remnants at anthesis are somewhat larger than the above-mentioned residues but are still small, formless, and difficult to identify (fig. 9). Markedly different is the appearance of a degenerate egg apparatus with a filiform apparatus; it is larger, structured, and, therefore, easier to identify. The different appearance is accentuated by the varied extent of deformation, contrasting with the uniform look of the prefiliform apparatus degeneration.

Abnormalities

TWIN EMBRYO SACS.—This phenomenon was originally reported as very rare (SCHROEDER 1951, 1952) but later was found in considerable numbers in some cultivars (TOMER et al. 1976). We have observed

[2] Further on "degeneration" will refer only to the micropylar site of the embryo sac. Degeneration of the antipodals will be ignored, being a normal development in avocado (SCHROEDER 1952; TOMER et al. 1976) unrelated to fertilization. Degeneration of the polar nuclei is rather infrequent (TOMER et al. 1976).

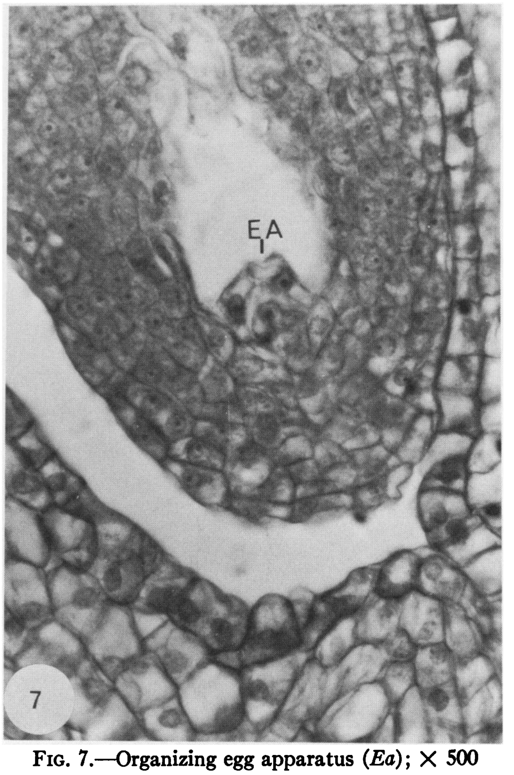

FIG. 7.—Organizing egg apparatus (*Ea*); × 500

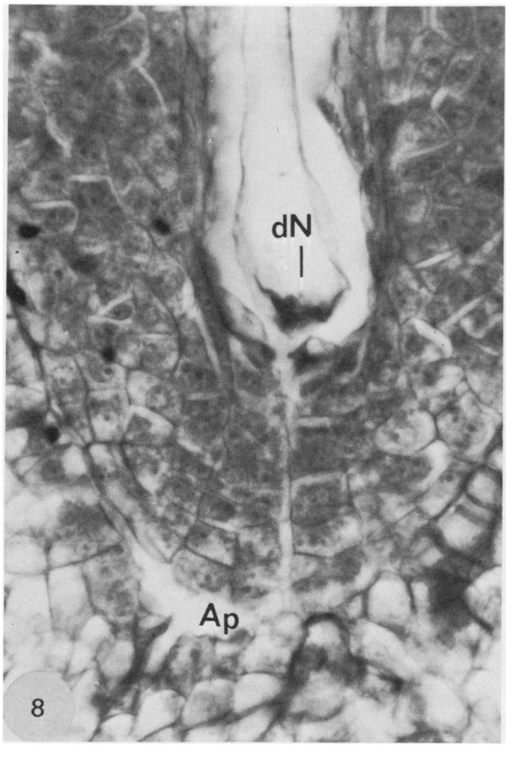

FIG. 8.—Degenerate micropylar nuclei (*dN*) of four-nucleate embryo sac; × 550.

twin archespores and twin megaspore mother cells not in a row but side by side (fig. 10), indicating the possible origin of a twin embryo sac (fig. 11).

MISSING EMBRYO SAC.—This abnormality was found in very large proportions in some cultivars (TOMER et al. 1976). Its origin could be traced back to the nucellus primordium, since ovules which could be expected to have an archespore or a megaspore mother cell were lacking. This is a plausible reason for a missing embryo sac but does not exclude the possibility of later origin. A degenerating archespore or megaspore mother cell also might give rise to a nucellus lacking an embryo sac, one degenerate cell not being discernible in a growing and differentiating nucellus. Still, a degenerating archespore or megaspore mother cell was not seen.

CAVITIES, COMPARTMENTS, IRREGULAR NUMBER OF NUCLEI.—Instead of the normal embryo sac in maturing ovules, not infrequently a compartmentalization within the nucellus can be seen taking on two appearances, one of which was termed "cavities" (TOMER et al. 1976). These are two to four relatively large holes, variously positioned, oriented in different directions, and sometimes separated from one another by large masses of nucellar tissue. Cavities contain densely clustered nuclei in about the same number as in the normal embryo sac (figs. 12, 13). The other type of compartmentalization might be related to the phenomenon described by SCHROEDER (1952) as "cross-walls." The space normally occupied by one embryo sac is divided into two to four compartments,

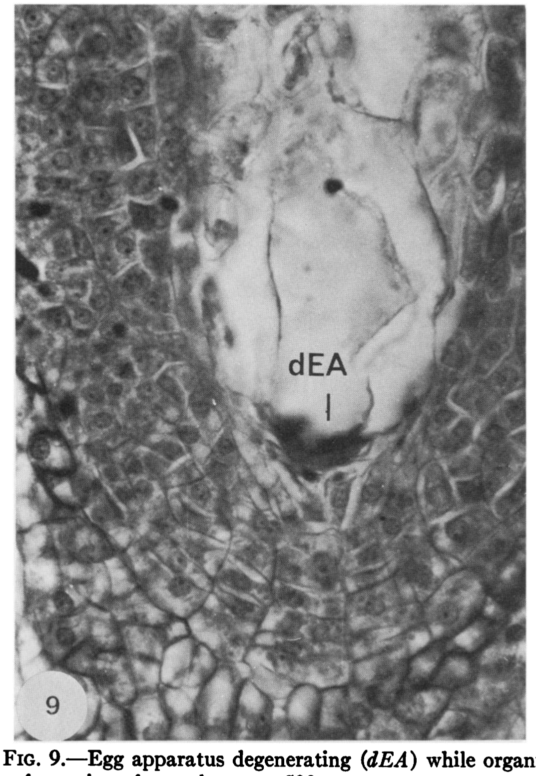

FIG. 9.—Egg apparatus degenerating (*dEA*) while organizing; formation of cytoplasm; × 500.

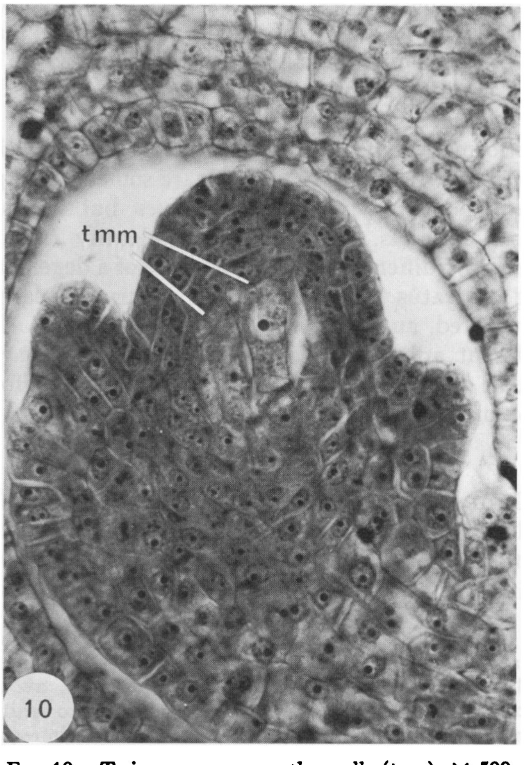

FIG. 10.—Twin megaspore mother cells (*tmm*); × 500

separated from one another by thin cytoplasmic membranes (walls) staining with fast green. They sometimes resemble large cells containing one or two cytoplasm-embedded nuclei, but frequently they are of long, narrow, and irregular shape containing a few nuclei. Their long axis is parallel to that of the nucellus. The compartment at the micropylar region is sometimes a normal embryo sac of reduced size. Both types may occur together in one ovule and thus increase the number of compartments (fig. 14).

Discussion

Since degeneration of the embryo sac may occur at different stages of ovule development, commencing with the four-nucleate stage, the resulting appearance at anthesis will be very much determined by each stage. Thus, the most extreme forms of degeneration might originate from the earliest breakdown. Therefore, an attempt to classify the variable appearance of degeneration should include a consideration of the origin of degeneration and should not be based

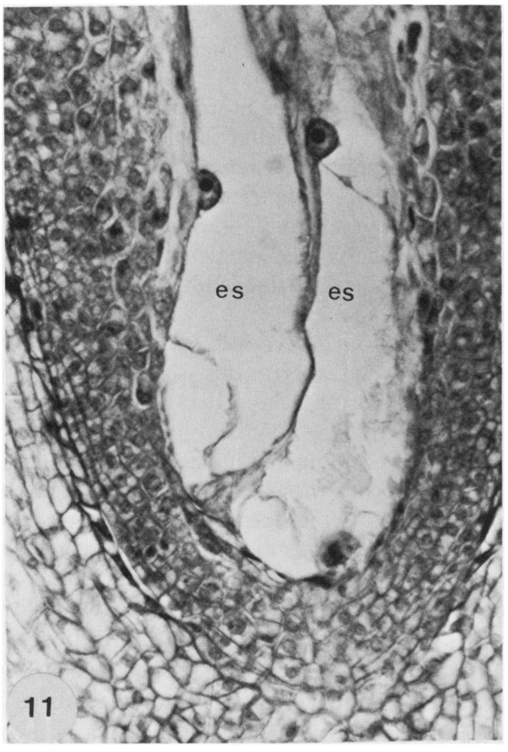

FIG. 11.—Twin embryo sacs (*es*); × 300

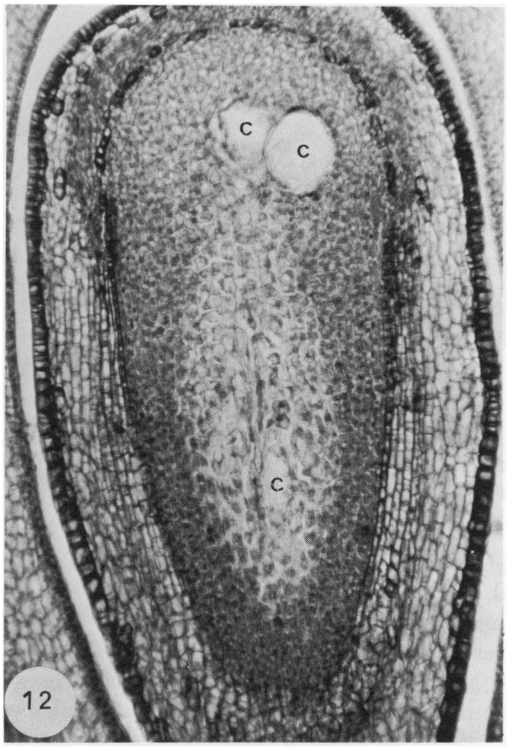

FIG. 12.—Cavities (*c*) in nucellus; × 125

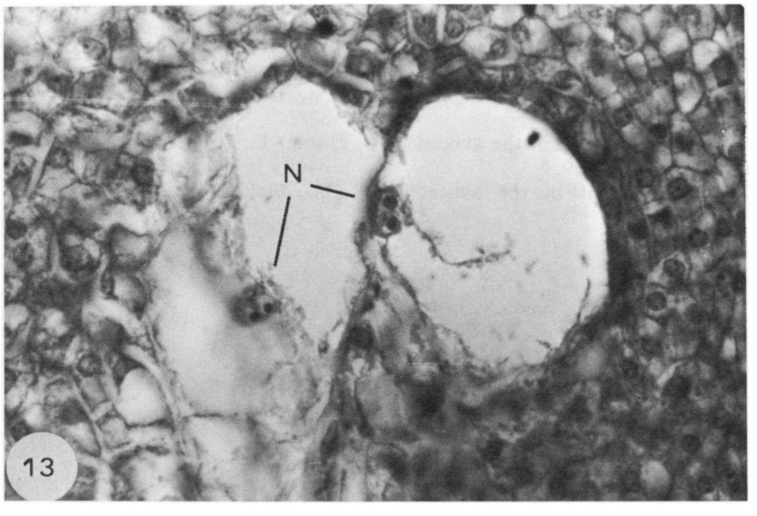

FIG. 13.—As fig. 12; the cavities contain clusters of nuclei (*N*); ×500

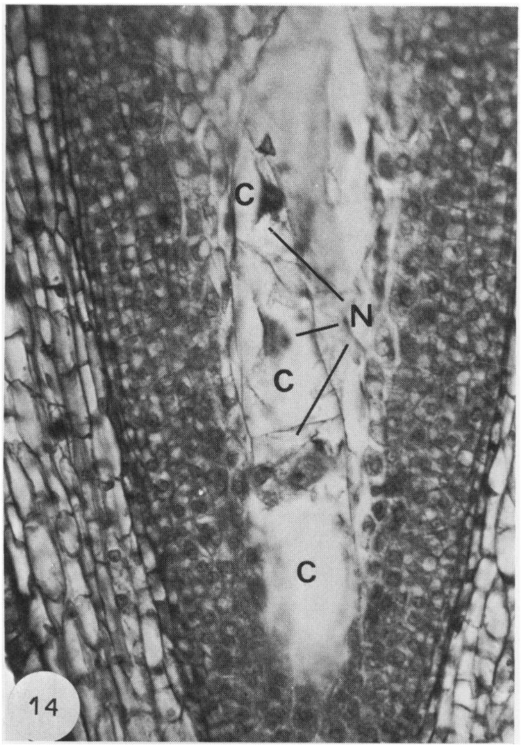

FIG. 14.—Compartments (*C*) in the micropylar region containing nuclei (*N*); this ovule contained also a small, degenerate embryo sac (*dEA*) in a nearby region; × 300.

wholly on the measure of deformation (TOMER et al. 1976).

Compartments, cavities, and irregular numbers of nuclei may have a common explanation if we assume that they originate from a tetrad of megaspores behaving abnormally; that is, the three micropylar megaspores (or some of them), instead of degenerating, continue to develop and, together with the chalazal megaspore, form the compound contents of the nucellus. This assumption opens the way for the many combinations which actually are encountered when large numbers of ovules are examined. Essentially, the cavities and compartments are embryo sacs competing for space and are reduced in size. The abnormal shape, the irregular number of nuclei they contain, and the unorganized behavior of the nuclei may be the result of a loss of the normal control mechanism. The evidence for these assumptions has to be worked out. In contrast to this behavior is the perfectly normal appearance of twin embryo sacs which we assume arise from twin archespores via twin megaspore mother cells.

Special mention must be made of the dots in the apex of the nucellus (fig. 6). A nucellar apex is rarely mentioned in the literature. JENSEN (1965, 1969) described a nucellar apex for cotton and pointed to the specialization of these cells. The dots in our study were confined to the apex cells during a limited phase of ovule development and may indicate the special physiological nature of the apex cells. No reference to these dots was found in the literature.

In our study of the avocado ovule we encountered a great variety of aberrations, some of which were described by TOMER et al. (1976). Some variations, apart from artifacts, were easy to comprehend, but the picture was frequently unclear or unintelligible. We tried to clarify these cases by tracing them back to their origins, which resulted in a better understanding of the various kinds of degeneration. In an attempt to sort the manifold appearance of avocado ovules, we have divided them into the following classes: no embryo sac; immature embryo sac because normal development stopped at some early stage; normal embryo sac; degenerate embryo sac; twin embryo sacs originating from twin archespores; and compartments and cavities originating from megaspores.

LITERATURE CITED

JENSEN, W. A. 1965. The composition and ultrastructure of the nucellus in cotton. J. Ultrastruct. Res. **13**:112–128.

———. 1969. Cotton embryogenesis: pollen tube development in the nucellus. Can. J. Bot. **47**:383–385.

SCHROEDER, C. A. 1940. Floral abnormality in the avocado. Yearbook Calif. Avocado Ass. **1940**:36–39.

———. 1951. Flower bud development in the avocado. Yearbook Calif. Avocado Soc. **1951**:159–163.

———. 1952. Floral development, sporogenesis, and embryology in the avocado, *Persea americana*. BOT. GAZ. **113**:370–378.

TOMER, E., M. GOTTREICH, and S. GAZIT. 1976. Defective ovules in avocado, *Persea americana*. J. Amer. Soc. Hort. Sci. **101**(5):620–624.