A developmental and histochemical study of the female reproductive system in Ornithogalum caudatum Ait. using light and electron microscopy

Varien Russell Tilton
Iowa State University

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A DEVELOPMENTAL AND HISTOCHEMICAL STUDY OF
THE FEMALE REPRODUCTIVE SYSTEM IN
ORNITHOGALUM CAUDATUM AIT. USING LIGHT AND
ELECTRON MICROSCOPY. (VOLUMES 1 AND 2)

IOWA STATE UNIVERSITY, PH.D., 1978
A developmental and histochemical study of the female reproductive system in *Ornithogalum caudatum* Ait. using light and electron microscopy

by

Varlen Russell Tilton

Volume 1 of 2

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1978
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PREFACE

"...see what everybody else has seen, and think what nobody else has thought."

Dr. Albert Szent-Györgyi

At the outset of this adventure, I had intended to investigate the anatomy of ovule initiation, megasporogenesis, and megagametogenesis. This, with the hopes of being able to deduce some sort of heretofore, unthought-of brainstorm as to the functional role of the various parts. Well, for one reason or another, this was not to be. The original plan has blossomed into a look at the entire female reproductive system, the growth of pollen tubes from stigma to ovule, and some of the physiological reactions of the flower in response to germinating pollen and incipient fertilization.

When I began to really rummage around in the Iowa State University library, I was captivated by the literature available there. Going beyond books and review papers back to the original papers, many from the 19th Century, was most satisfying. It made me realize that many of the ideas which I had thought I was so clever in dreaming up on my own had been around for a long time. I was also greatly impressed with many of the illustrations by the early Germans and Japanese, but particularly the Germans. Well anyway, the point is that in order to give credit where credit is due, I had to dust off a few volumes in the library. I was really amazed and feel greatly rewarded by the experience.

So there I was amidst all that wonderful knowledge, and I figured that I had best take advantage while I had the chance. What better
opportunity to try and understand the nature of the flower, in its entirety, as a biologically functional reproductive system? In my attempt to accomplish this task, I necessarily had to include some literature not directly pertinent to my own researches; but, in order to understand the whole, one must know very well all its parts.

To understand all aspects of the flower and the reproductive process with equal clarity would require a lifetime of study and research. I have only completed page one. I look upon this dissertation as a foundation for whatever I may accomplish a la mañana. It has afforded me the opportunity to put all of my thoughts on the matter, correct or otherwise, into a coherent (I hope) text, and has provided me with ideas for future research.

The quest for my doctoral degree has at times been a royal pain in the ass—some of the courses and jerk instructors; the hours of toil and sweat with the dictionary and that German grammar; the frustration of running out of swearwords when things went wrong in the lab; the picnics that never got picnicked; the beer that somebody else drank; my beautiful bride whose company I never got to enjoy....

But, there have been rewards too—I was really tickled to get into some of the original German papers; some of the courses were excellent and the instructors a truly stimulating influence; and my own research has been very satisfying to me.

Has it been worth it all? I don't know yet—I don't think I will be able to answer that question for several more years.
INTRODUCTION

Although gynoecia from a wide variety of plants have been studied in part and parcel by many botanists, there are only a very few studies which include the development and structure of the entire gynoecium from any given plant. In this paper, the gynoecium as a whole, and all of the tissues which comprise it, have been investigated with both light and electron microscopy. In addition, pollen tube growth and some of the physiological responses of the flower to pollen germinating on the stigma and incipient fertilization were investigated.

Most of the literature on carpels deals with floral morphology for theoretical, phylogenetic, and taxonomic purposes, types of placentation, and vascular patterns. There also has been some work done on carpel initiation and carpel fusion, but there is relatively little information on carpel anatomy per se. In particular, there is a great lack of ultrastructural information. One of the aims of this study, then, has been to follow the development of the carpels in Ornithogalum from an anatomical viewpoint. This proved to be very interesting, and the carpels were found to be a great deal more complex than first thought.

As with the carpels, the structure and function of styles has been taken pretty well for granted. In only a very few plants has the style been studied with electron microscopy. As of this writing, the list consists of just five dicots and only one monocot; a rather appalling lack of knowledge about such an important organ. In this study, stylar development was followed through the entire sequence of events in Ornithogalum. It was indeed a most interesting phenomenon to observe.
The heart of this study was to follow the development of the ovules. Although the list of plants from which ovules have been studied ultrastructurally is a bit longer than that for carpels and styles, there is still an amazing paucity of knowledge about the ultrastructure of ovules. In particular, there is very little ultrastructural information on mei- sporogenesis and megagametogenesis. The mature egg apparatus has been studied in only some fifteen species of flowering plants. This number falls drastically when other features of the ovule are considered and, in some instances, falls to zero. Examples of the latter case are hypostases, cells lining the micropyle, and obturators. These tissues have never before been examined with transmission or scanning electron microscopy. And, by the same token, in no plant has the entire developmental sequence of ovule ontogeny been studied ultrastructurally.

The principal aim of this dissertation, then, has been to examine the complete developmental process of the entire female reproductive system in one plant and see how the various parts are integrated to form a biologically functional system. Due to time considerations, some technical problems with specimen preparation, and my personal interests, not all aspects are covered in equal depth.

The significance of this dissertation is twofold. First, it adds new information to the existing paucity of knowledge on carpels, styles, and ovules--new information from which some new ideas have evolved concerning the processes and events surrounding sexual reproduction in flowering plants. And second, the work in this study has provided a large part of the background information which I will need for research I plan to do in the future. It has given me a feeling for the kinds of
things that will need to be done to obtain the facts not yet known about sexual reproduction in angiosperms, and ultimately to learn how to understand and possibly control this process for the benefit of humankind.

This study, then, is a comprehensive investigation of gynoecial development in *Ornithogalum caudatum* Ait., a member of the tribe Scilloideae in the lily family according to Hutchinson's (1959) classification scheme. This particular plant was selected for three principal reasons: (1) it flowers year-round in a greenhouse, (2) it has an indeterminate inflorescence, and (3) it is readily available.

References to the Genus *Ornithogalum* date back to the time of the ancient Greeks and the writings of Theophrastus in 300 B.C. In fact, the name, *Ornithogalum*, is thought to be of Greek origin and translates as bird's milk from the original Greek. The reference to milk is probably due to the creamy color of the blossoms, especially when they first open (Leighton, 1944a). *Ornithogalum* is also referred to in the Bible as dove's dung in II Kings, chapter 6, verse 25. The "white specks" over the countryside are probably the bulbs of *O. umbellatum* L. which are still eaten in Palestine today (Leighton, 1944a).

*Ornithogalum* goes by several common names, the most prevalent one being Star of Bethlehem in obvious reference to the stellate appearance of the flowers and their abundance in the Middle East. Other names include Bird's Milk, Vogelmilch (= bird's milk) by German authors, Milk Stars, and Cape Snowdrops. The latter name is most likely due to the fact that many of the species are native to the Cape region of South Africa.

The genus is thought to have had its original centers of evolution in Central and South Africa, Europe, and Western Asia. Its range now
covers most of Africa, most of Europe (except the northern extremes), the Mediterranean, and Western Asia (Zahariadi, 1962, 1965). Ornithogalum is presently actively evolving and undergoing intense speciation in the Mediterranean and Middle East (Zahariadi, 1965; Cullen and Ratter, 1967). Ornithogalum caudatum, the species of interest at hand, is of South African origin (Leighton, 1945; Feldman et al., 1975).

The genus has undergone several major taxonomic investigations (Leighton, 1944a,b, 1945; Neves, 1959; Zahariadi, 1962, 1965; Pienaar, 1963; Cullen and Ratter, 1967; Krasnova, 1970) of which the papers by Leighton (1944a,b, 1945) and Zahariadi (1962, 1965) are the most significant. In addition to these larger works which I have listed, there are many short, species-specific notes, far too numerous to mention here.

Ornithogalum, particularly O. virens, has been a popular experimental genus with various cytologists: Therman (1951)—chromosomal attractions during cell division; Czapik (1966, 1972)—cytological differentiation, cytoembryology; Karagozova and Van Khankh (1972)—endomitosis in embryo sac cells; Church (1973)—nucleic acid synthesis during meiosis; Schweizer (1976)—microtechnique-fluorescence of chromosomes; and Ravindran (1977)—homologous chromosome associations in somatic cells. Ornithogalum caudatum is used as a tool in teaching cytological techniques in the graduate microscopy class (Botany 680) of the Department of Botany and Plant Pathology at Iowa State University.

The genus has also been looked at anatomically: Raciborski (1895) and Węycicki (1929)—oleoplasts in epidermal cells; Samson and Karstens (1971)—bulbs and bulbils; Belyanina and Krylova (1971)—epidermis; Scannerini (1972) and Bonfante and Scannerini (1975)—endomycorrhizal
associations. Histochemical works include those of Kwiatkowska (1966, 1971a,b, 1972a,b,c, 1973a,b) - lipotubuloids; and physiological papers include those of Goss (1962, 1963, 1968) - pollen nutrition and germination; Belyanina and Krylova (1970) - developmental rhythms; and Feldman et al. (1975) - thermostability of four characters.

Of more pertinent interest to the paper at hand are the studies on the embryology of the genus. Although several of the previously noted papers have some embryological information, such information generally consists of a passing remark as to the type of placentation, type of embryo sac development, or type of endosperm development, and are thus of no further value here. The following papers, however, are of value and will be referred to later when appropriate. To my knowledge, the earliest investigations were those of Dalmer (1880), who looked at pollen tube growth in two species, and of Raciborski (1893b, 1895), who looked at the ovules (1893b) and the carpels (1895). Other embryological papers of import on the genus are those by Schnarf (1928), Wunderlich (1937), Desole (1947), Stenar (1951), Chiappini (1962), Czapik (1966, 1972), Karagozova and Van Khankh (1972), and żabińska (1972).

As a note of interest, in that the anomaly has been noticed in the genus *Ornithogalum*, Geitler (1941) found that some anthers of *O. nutans* contain abnormal pollen grains which upon germinating give rise to an embryo sac. In addition to *Ornithogalum*, other plants have been shown to have this anomalous character. It was first reported by Němec (1898) for *Hyacinthus*, and then by Mol (1923, 1933), Stow (1934), and Naithani (1937), all with *Hyacinthus*.
In regard to economic considerations, the genus as a whole is rather insignificant, but there are some species of commercial value. The bulbs of *O. umbellatum* are used for food in Palestine, and *O. thyrosoideae* and *O. lacteum* are exported from South Africa to Great Britain and the United States as floricultural products (Leighton, 1944a).

Several species are reported to be toxic to livestock: *O. saundersiae* (Quin, 1927); *O. longibracteatum* (Mettam, 1930); and *O. thyrosoideae* (Leighton, 1944a). *Ornithogalum pretoriense* was originally thought to be toxic to sheep, but was later shown to be harmless (Van der Walt and Steyn, 1946). The underground parts are more poisonous than the aerial, and they are most toxic prior to floral abscission (Mettam, 1930). The toxins are very specific for the digestive mucosa (Quin, 1927). Of a somewhat different nature, the cell sap of *O. caudatum* contains calcium oxalate raphide crystals and causes a type of contact dermititis in man (Pohl, 1965).

Two species are known to be alternate hosts for rusts: *O. pyrenaicum* for *Puccinia hordei* (Dennis and Sandwith, 1948) and *O. umbellatum* for *P. simplex* (Dupias, 1947). The genus also has a virus named after it, the *Ornithogalum* mosaic virus (*Marmor scillearum*) which is transmitted by two insect vectors, *Aphis* and * Macrosiphum* (Smith and Brierley, 1944).

Several species of *Ornithogalum*, e.g., *O. colchicum*, produce copious amounts of colchicine and are grown commercially as a source of the drug. Colchicine has been shown to be present in all organs with the most being produced in the flowers and seed coat, and the least in the endosperm and roots (Klein and Pollauf, 1929).
During the late 1950's and early 1960's, Ornithogalum was rather intensely investigated as a source for other drugs. Ornithogalum umbellatum was found to be a good source for drugs used in the treatment of heart disease (Mrozik et al., 1959; Vogelsang, 1961). This species has eight digitaloid glycosides (Mrozik et al., 1959) which are effective substitutes for digitalis in treating congestive heart failure (Vogelsang, 1961). The genus is also a source of several hemaglutinins, some of which are found in small amounts in the seeds of O. caudatum (Piette and Parvanchère, 1962). Research on extracts from Ornithogalum species continues today and the interested reader is directed to the Biological Abstracts or other appropriate sources for further information.
Inflorescences and Floral Morphology: An Overview

Inflorescences have been classified on the basis of two factors: position and the timing or sequence of anthesis. On the basis of the order of development of flowers within a cluster, inflorescences are classified as determinate (cymose) and indeterminate (racemose). In determinate inflorescences, development of the first flower limits apical growth of the main axis of the inflorescence, and the sequence in development of the other flowers is basipetal. In indeterminate inflorescences, the first flower is the lowest, and the order of the development of the others is acropetal. The number of flowers in indeterminate inflorescences is theoretically unlimited. Basipetal order of development is often called centrifugal, and acropetal development, centripetal. Many inflorescences are intermediate in type, especially the racemose forms that have a terminal flower.

On the basis of position on the stem, inflorescences are grouped as terminal, axillary, and intercalary. Strictly terminal clusters terminate branches while axillary clusters are terminal on short axillary branches or represent foliaceous axillary branches which have been reduced to inflorescences. Intercalary inflorescences are terminal clusters which have been left behind by continued apical growth of the main axis. This results in alternating fertile and sterile sections. Intermediate conditions may exist with this scheme also, i.e., cauliflory.

The flower is often considered to be characteristic of angiosperms yet no one character can be used to set these plants apart from other
seed-bearing taxa. Moreover, the flower cannot be so defined as to separate it from similar reproductive structures of the gymnosperms. The morphological basis of the flower rarely is emphasized in definitions, and often it is called the reproductive structure of the angiosperms. Morphologically, it is a determinate stem tip bearing sporophylls and, commonly, other appendages that are sterile. This definition, however, applies equally well to many cones, including those of gymnosperms and even some of the lower vascular plants (Eames, 1961).

The term, gynoeclum, as originally defined by Roeper (1826) includes the entire female reproductive system, and is used as such in this dissertation. In agreement with Parkin (1955) and Eames (1961), the term, pistil, as applied to gynoecia is undesirable and its use will be avoided. [For more detailed discussion of the origin and application of the terms, androecium and gynoeclum, the interested reader is directed to Roeper (1826), Church (1919), and Parkin (1955).] According to classical interpretation of the gynoeclum, the basic unit of gynoecial construction is the carpel (Guédés, 1972). Carpels are defined by Sattler (1974) as folded megasporophylls, i.e., each is a "...folded phyllome bearing and enclosing ovules." This definition leaves no doubt as to the morphological and evolutionary origin of carpels.

The idea that carpels and all other floral parts may be compared with leaves is as old as botany itself, but, in 1790 with his Attempt to Interpret the Metamorphosis of Plants, Goethe was the first to compose an effective formulation of the foliar theory. Johann Wolfgang von Goethe, a German poet, is probably more noted for his poetry and his authorship of Faust than he is for the several volumes he wrote to record his natural
observations. Most of his scientific writings were zoological in nature, but his essay on the metamorphosis of plants has had the greatest effect on scientific thought. Goethe also coined the term, metamorphosis (Eyde, 1975).

Goethe believed that all plant appendages are variants of an intuitively perceived ideal appendage, the primal leaf, which somehow contains all of its own transformations.

"In the organ of the plant which we are accustomed to call the leaf lies the true Proteus who can hide or reveal himself in all vegetal forms. From first to last, the plant is nothing but leaf..." (Goethe, 1790, as cited by Eyde, 1975).

He further believed that all flowering plants are variants of an Urpflanze, or plant archetype (Eyde, 1975).

The foliar theory was developed independently and in a more rational context by the famous French botanist, Augustin-Pyramus de Candolle. De Candolle introduced his first version of the theory in his Elementary Theory of Botany in 1813 (Eyde, 1975). Although the foliar theory was first put forward in a coherent context by Goethe, Goethe was not trained as a scientist and consequently his theory was incomplete (Arber, 1937). De Candolle later became aware of Goethe (as did Goethe of de Candolle), but it was de Candolle who put the foliar interpretation on a firmer basis.

Goethe and de Candolle were of greatly different convictions and this difference in attitude was expressed in their writings. To Goethe, Newtonian science was a threat. In contrast, de Candole believed wholeheartedly in the exact sciences and sought to build botany along the same
lines. Both men were devoutly religious and both wrote poetry, but de Candole kept religion and poetry out of his scientific works (Eyde, 1975). In his Vegetable Organography, de Candole (1827) incorporated some of Goethe's ideas, and he went on to further refine and expand the foliar theory (Arber, 1937; Eyde, 1975). It is this refined version of 1827 by de Candole which yet serves as the basis for classical morphological interpretations of the flower.

A few other notable morphologists who have either contributed to, or who have supported, the classical foliar theory include: Van Tieghem (1875) who established the appendicular nature of the entire carpel (in the sense that the leaf is appendicular); Arber (1937); Wilson and Just (1939); Bailey and Swamy (1951); Eames (1961); Foster and Gifford (1974); and Sattler (1974).

There have been numerous attempts to refute this theory, and among the more notable is a series of papers which appeared in the 1930's. H. Hamshaw Thomas (1931, 1934) was a proponent of the New Morphology school which believes that carpels originate from a branch structure by evolutionary stages totally different than those passed through by the foliage leaf. Hunt (1937) also supported this view. Both structures, however, are regarded as originally derived from specialized branches of the thallus. The ovules are held to represent original terminal structures, the placentae separate branches, and the carpel wall a cupular structure which is quite distinct in origin from a typical foliar structure.

Thomas thought that the pteridosperms were the ancestors of the angiosperms, and, according to him, the ancestors had three-dimensionally
branched fertile structures. Ovules and pollen sacs were borne more or less terminally on these branches. Thus, Thomas opined that the precursors of the carpels and stamens were not flat, leaflike structures as postulated in classical theory.

A contemporary of Thomas, J. McLean Thompson (1933, 1934) proposed a theory of apocarpous angiospermy. According to Thompson's Theory of Scitaminean Flowering (1933), the ancestral reproductive apparatus was a stem with fertile segments but with no appendages. He considered the carpels as emergences of this fertile axis which appear leaflike in some groups of plants because of evolutionary specializations. In regard to carpels, he concluded "...carpels as historic documents in the story of angiospermy are considered unnecessary" (Thompson, 1934).

Another author of this era with other than classical views was Edith Saunders. Although Saunders' view of the flower was basically foliar in nature, she interpreted almost every longitudinal vein in the seed bearing part of the flower as a vestigial carpel. According to Saunders, then, the peanut is a 10-12 carpellate structure (Eyde, 1975) -- a rather amazing accomplishment for the legume!

The most noted extant member of the New Morphology is probably A. D. J. Meeuse. In his Anthocorm Theory, Meeuse advocated the former existence of complex fertile branched structures which he termed anthocorums. He then modified and combined these hypothetical structures in various ways to suggest the stages through which various kinds of flowers evolved in a number of different ways a number of times (Eyde, 1975).

In concluding this very brief summary of theories on floral morphology, I would like to quote a passage from Eames (1961) and one from
Sattler (1974). I include these passages because of their bearing on morphological research, and also because I believe that the principle that they convey applies to all investigations in the life sciences, and probably to other sciences as well. Stated quite simply, the principle is that one cannot take an isolated entity out of context and explain, or even pretend to understand, the whole.

Eames, 1961—"That the flower is only in part a mature structure is commonly overlooked; the receptacle and carpels are still in early stages when the rest of the flower is mature. Failure to recognize that the vascular supply of the carpels within the receptacle is incomplete at flowering time has led to incorrect interpretations...."

Sattler, 1974—"A final remark is necessary about the importance of developmental studies in morphology as it relates to phylogenetic considerations. ...ontogenetic data are of little importance in this respect (Carlquist, 1969). This tenet is not acceptable.... First ...evolution is not a transformation of mature structures, but a succession of modified ontogenies. How then, can one understand and reconstruct phylogeny by excluding the continuity that occurs during ontogenies and links temporally the mature stages. Second, evolutionary changes do not occur in mature structures, but through processes that affect ontogenies and--as a result--change mature structures. Third, if one could deduce ontogeny from a knowledge of the mature structure, developmental studies would not be needed. We know, however, that such a deduction is not necessarily possible. Even the same or very similar structures may sometimes develop differently. Fourth, even efficiency considerations so much emphasized by Carlquist (1969) may require a knowledge of developmental processes because economisation occurs by a change of developmental processes.... It seems that development is relevant to almost everything, and this is not surprising because an organism is not just a mature form, but exists in space and time. The mature form is only one time slice of its space-time extension."

The Gynoecium: General Considerations

Having covered some of the theoretical aspects of the flower, some general considerations of the gynoecium now will be considered. The
carpel, as a simple, folded, or involute laminar appendage, with one to many ovules, varies greatly in form. It may be stipitate or nonstipitate; completely or incompletely sealed. The carpel may be simple, without differentiation into fertile or sterile parts, e.g., woody Ranales, or it may be complex. The complex form is divided into a proximal, ovule-bearing part, the ovary; a distal, pollen-receptive part, the stigma; and a median section, the style. The carpels may be free from one another producing an apocarpous gynoeclum, or they may be connate to varying degrees. Fused carpels give rise to syncarpous gynoecia (Eames, 1961).

Connation among carpels may extend from base to tip or for only part of their length. Fusion of ovaries alone is common, and by just the proximal parts of the ovaries is frequent. Fusion of stigmas alone is rare, but does occur in some families—Apocynaceae, Asclepiadaceae, Rutaceae, and Simarubaceae. Free styles and stigmas often form a prominent part of syncarpous gynoecia. The fusion of parts may be ontogenetic or congenital, or it may be that the distal parts fuse ontogenetically while the basal parts have fused congenitally (Baum, 1948; Walker, 1975b).

Where lateral union between carpels is incomplete, the unfused areas commonly become secretory, and well-defined nectaries are formed. These nectaries are common in the Liliaceae (Eames, 1961; Sterling, 1972, 1973b,c, 1974a,b, 1975) and related families and occur occasionally in the palms and other genera (Eames, 1961; Uhl and Moore, 1971). The secretions of these glands may exude along longitudinal slits between carpels or at the top of the ovary when the carpels are fused along the dorsal margin. These nectaries may have elaborate form and exude the
secretion through a specialized canal leading to a small opening on the
top or side of the ovary as in Cocos and Ananas. The septal type of
nectary seems to be restricted to monocots and has probably arisen inde­
pendently in members of this taxon.

The presence and position of the unfused areas of carpel walls in
syncarpous gynoecia is related to the history of carpel connation. The
transformation of the semi-enclosed pockets between the carpels into nec­
taries and the elaboration of their form constitute an excellent example
of adaptation of flower structure to insect pollination.

The closing of the carpel comes about, structurally, by an upturning
of the sides of the lamina and the bringing together of the adaxial sur­
faces or edges with more or less complete fusion. The carpel has appar­
tently closed independently in many lines and in a variety of ways. A
primitive method of closing is that considerable portions of the adaxial
surface come together. This forms the conduplicate type of carpel, and
has been considered the basic type from which all others have been
derived. A survey of many families, however, suggests that this method
is only part of the history of carpel closure. Carpels in which the
lamina sides appear rolled upward and inward are known as involute, and
it seems improbable that all types of involute carpels have been derived
from the conduplicate form (Eames, 1961).

The pattern of ovule arrangement on the carpel constitutes placenta­
tion, and the placenta has been variously defined as "the swollen margins
themselves" (Puri, 1964) and the "...area where the ovules are attached,
usually more or less enlarged as an emergence of the laminar tissues..." (Eames, 1961). In primitive taxa, there may be little or no modification
of the region where the ovules are borne. In these groups, the placenta is merely a location. Absence of an emergent placenta is probably the primitive condition. The ovule-bearing projection may be simple, a mere low cushion or ridge, but it is frequently large and complex in form and structure. It may form a major part of the ripe fruit and even project from the ovary wall.

There are several views as to the morphological nature of the placenta: (1) placentae in all angiosperms are axial; (2) placentae are not of the same nature in all cases; and (3) placentae in all angiosperms are carpellary. The term axile should be distinguished from axial. Axial infers that the ovules are borne on the floral axis in the sense that the axis is the shoot itself (Puri, 1952). In classical terminology, axile (Van Tieghem, 1891) or marginal-axile (Sachs, 1882) infers that the ovules are borne on the carpels along the axis of the ovary in the sense that the axis is an imaginary longitudinal line through the center of an organ. Ovules are thus also carpellary in origin, vice axillary (Eames, 1961; Puri, 1964). Although some ovules may appear to be terminal on the receptacle, their "...anatomy demonstrates that there are no cauline ovules in the angiosperms" (Eames, 1961).

That the placenta is axial in nature was formally suggested by Saint-Hilaire (1816). He stated that the floral axis, after producing the carpellary leaves, continues up and bears ovules which may be borne on the central column or on its branches. This view was more emphatically championed by Schleiden (1849) who regarded ovules as being modified buds, which, as a rule, are borne on axial parts (Puri, 1952). The view that the placenta is of carpellary origin was formulated by Robert Brown.
(1816) and has since been incorporated into classical theory.

Although the marginal position long has been accepted by most as the primitive ovule position, the term, submarginal—now commonly used, was applied to this type of placentation as early as the mid 1800's but did not continue in use. The critical study of the highly primitive carpels of the woody Ranales together with ontogenetic studies in many taxa, have demonstrated that the primitive position of the so-called marginal ovules is submarginal and marginal is morphologically incorrect. The submarginal position is characteristic for most taxa with free carpels while the laminar position is found only in primitive families. Several taxa show the transition from laminar to submarginal placentation demonstrating that the submarginal condition is derived, e.g., Degenariaceae, Winteraceae, Nymphaeaceae, Cabombaceae, and Ceratophyllaceae (Eames, 1961).

According to Dickison (1968), the basic characteristics of primitive gynoecia include: (1) complete or nearly complete apocarpy; (2) visible conduplicate folding; (3) open ventral sutures; and (4) large numbers of ovules. In contrast, evolutionarily advanced gynoecial features include: (1) reduction in carpel number to one; (2) differentiation and vascularization of a narrow, elongate style; (3) formation of restricted, terminal, often peltate stigma; (4) reduction in ovule number to one; (5) varying degrees of basal adnation and/or lateral concrescence; (6) complete fusion of ventral suture; (7) reduction or amplification of basic tri-veined vascularization; and (8) tendency toward involute closure. In considering these two lists one should remember, as pointed out by Eames (1961), that evolutionary changes in these characters have progressed unequally.
Therefore, an advanced character may be accompanied by one which is primitive.

The primitive features are exemplified best among the woody Ranales, of which the genus **Drimys** is probably the most celebrated. **Drimys piperita** Hook f. has the least modified carpel form of extant species (Bailey and Swamy, 1951). At anthesis the carpel is stipitate and has relatively thin, conduplicately folded lamina. There is no style, and the stigmatic papillae are widely distributed over the inner surfaces and free margins of the carpel. At times these papillae extend outward onto its more exposed outer surfaces. The carpel thus has two external, independent, stigmatic crests (Bailey and Swamy, 1951). The ovules are initiated closely temporally, but the uppermost and lowermost on each placenta may lag behind the others in development. Ovules are supplied by the ventral veins with occasional branches from the dorsal connecting with the ventrals at a later stage (Tucker, 1975).

A few other papers of note dealing with the gynoecia of primitive taxa in a more or less general manner include: Canright (1960)—Magnoliaceae; Dickison (1968)—Dilleniaceae, and (1975)—Cunoniaceae; Sampson and Kaplan (1970)—**Pseudowintera**; and Kaul (1976)—Alismatales. This is by no means an exhaustive list, and the interested reader is referred to the literature cited in Bailey and Swamy (1951), Sampson and Kaplan (1970), Dickison (1975), Tucker (1975), and Kaul (1976). Arber (1937), Wilson and Just (1939), Eames (1961), and Foster and Gifford (1974) are good starting places for those not familiar with this subject, as well as floral morphology in general.
There have been several morphological interpretations of the stigma and style, the most imaginative of which is probably that of J. McLean Thompson (1933, 1934). As part of his Theory of Scitaminean Flowering, Thompson (1933) believed that the stigma was derived from the tips of the male organs in his floral prototype, and that stigmas are potential anthers.

Thomas (1934) concluded that the stigma originated on the adaxial side of the carpel toward the base of the ovary and that it represents a specialized part of the inner ovary wall. He wrote, "The stigmatic surfaces of a single carpel together with the transmitting tissue represent the marginal part of the inner surface of the cupules lined with glandular hairs. The earliest stigmas arose near the lower part of the ventral side of the ovaries, but it is thought that, where carpels or ovaries were crowded together, natural selection would favor variation in which the stigma approached the free apex of the ovary."

Among some of the early classical interpretations were those of Brown (1840) who was probably the first to postulate a lateral origin of the stigma, citing Drimys as an example. Bailey and Swamy (1951) also support the view of a lateral origin. Working with Drimys, they concluded that the terminal position of stigmas has resulted from a reduction and reorientation of the external stigmatic crests found on primitive carpels such as these. In addition, they believed that a modification of the internal glandular surface has produced the transmitting tissue.

Another of the early workers, Capus (1878) investigated the path of pollen tubes in many plants and demonstrated the presence of a specialized
tract of tissue which conducts the pollen tubes from the stigmatic surface to the placenta. He concluded that the stigma is only the upper termination of this tissue which he called the conducting tissue. Arber (1937) agreed with Capus' findings and further stated that the stigma is not a definite morphological entity, a thesis also supported by Satina (1944). According to Zeisler (1938), however, the mature stigma can be delimited from the style, at least on the basis of its physiological response to hydrogen peroxide. In the presence of hydrogen peroxide, a mature stigma will react to evolve oxygen whereas the style will not.

The primitive carpels of Drimys have no style, only two external, independent stigmatic crests. Prior to anthesis, the space between the approximated surfaces of the carpels becomes occluded by a mat of interlocking glandular hairs. Pollen is held by the external hairs and the pollen tubes grow between the glandular hairs to the locule (Bailey and Swamy, 1951). Limnocharis flava also lacks a style, but the stigma is a hairless cleft which is open at anthesis (Kaul, 1976). Other primitive genera may have evolved a style but not a terminal stigma. Examples representative of the latter condition are Cercidiphyllum (Eames, 1961) and Hydrocleis nymphoides (Kaul, 1976) in which the stigmatic crests are decurrent on the style. In Hydrocleis, the style is open at anthesis and pollen grains are often found in the stylar canal (Kaul, 1976). Sahni and Johri (1936) reported finding pollen grains within the ovary of Tenagocharis latifolia, a condition which would preclude the necessity for the existence of a stigma. To my knowledge, this is the only report of such a condition, and, until careful photographic evidence can be obtained, this observation should be regarded with some caution.
Two-lobed stigmas seem to be primitive, as are some elongate stigmas such as found in many woody Ranales. The highly specialized stigma is either simple--globose or cylindrical--or it is elaborate in form--plumose or dissected. Greatly increased surface area, e.g., the elongate stigmas of many grasses, is an advanced feature associated with anemophily. A well-differentiated stigma, in contrast with the stigmatic crest, and a sealed carpel are characters associated with insect pollination (Eames, 1961). Small stigmas, as well as a reduction in the size of other floral organs, is characteristic of cleistogamous flowers (Harlan, 1945).

Morphologically, the style is usually considered to be the distal part of the primitive carpel with its ovules lost. Several families show a gradual transition from ovary to style with vestigial ovules and traces for lost ovules in the transitional region. Thus, the style in some taxa represents a sterile part of the ovary whereas in other taxa it seems to be formed by a secondary elongation of the distal part of the carpel. The style usually has a terminal position on the ovary, but, in some highly specialized taxa as the Labiatae and some of the achene-producing Rosaceae, the style appears to be lateral. Ontogenetic studies of these plants, however, have shown that the lateral position is secondary.

Hanf (1935) wrote what is probably the most widely accepted definition of the style. He defines a true style as the sterile paracarpous region of a coenocarpous gynoecium which is adapted to the conduction of pollen tubes and is more or less differentiated from the ovary. He stated further that apocarpous gynoecia never have true styles. Instead, they
have styloidia which are simple structures that possess a single, somewhat laterally situated vascular bundle.

Basing his classification scheme on the presence or absence of transmitting tissue and its degree of development in those styles which have it, Hanf divided styles into three categories. The open type has a stylar canal that is lined with an epidermis; the half-closed type, a canal lined with transmitting tissue; the closed or solid type, a canal completely filled with transmitting tissue (Hanf, 1935). The solid type lacks an internal epidermis and cuticle per se, and the cells are freely distributed in the center (Wilson and Just, 1939). Open styles are characteristic of monocots while half-closed and solid styles are more prevalent among dicots (Hanf, 1935). Guéguen (1901) stated that distribution of transmitting tissue in monocots is dependent upon ovule position and number.

According to Baum (1949), solid styles are the derived type, having arisen as an outgrowth of the carpel apex in the Leguminosae, and from the midrib of the lamina region in the Rosaceae and Caryophyllaceae. They may have arisen also by a secondary filling-in of the canal in other groups. Intermediate stages (half-closed) arose by carpel margins fusing, with or without a cavity remaining. Styles with free carpel margins were observed in primitive families only--Hamamelidaceae, Ranunculaceae, Magnoliaceae, Saxifragaceae, and others.

The tissue, or region of the style through which the pollen tubes grow on their way to the ovules was originally named conductor fructificationis (Hedwig, 1793, as cited by Dalmer, 1880). Since then, it has suffered a veritable plethora of new names: conducting tissue (Capus,
1878); stylar core (Arber, 1934); transmitting tissue (Harris, 1935); transmitting tract (Arber, 1937); inductive tissue (Iwanami, 1953); and stigmatoid tissue (Esau, 1960). Wilson and Just (1939), not to be left out, claimed that "A suitable functional designation for the whole region between ovary and stigma is 'pollen tube conductor'." As will be shown later, this designation falls short of the mark. Most of the other terms have been dropped from usage, and the two terms most commonly in vogue at present are transmitting tissue and stigmatoid tissue. I prefer the former, again, for reasons to be discussed later.

The transmitting tissue is commonly thought of as filling the stylar canal only, but it was shown in the 19th Century that this tissue may extend over the placenta and a considerable part of the ovary wall as well. Transmitting tissue within the style and ovary has been called the internal stigma, and is formed from superficial carpellary--rarely ovullary--tissue, from the epidermis, or from both epidermal and hypodermal tissues.

In dicots, transmitting tissue is usually papillose and reaches its greatest complexity in the Gamopetalae. The nonpapillose transmitting tissue of aquatics and some lower monocots probably represents a loss of papillose form in the cells. What is probably the most extreme form of the nonpapillose type is the multilayered degenerating tissue of the orchids. There are, however, several monocots which do have papillose transmitting tissue, e.g., some Liliaceae (Wunderlich, 1937). Both papillose and nonpapillose types commonly have chlorophyll distributed throughout the tissue (Eames, 1961).
The erroneous idea that collenchyma is present in the transmitting tissue is an old one. Guéguen (1901) wrote that transmitting tissue in most dicots, and particularly within the gamopetalous taxa, consists of from one to several strands of collenchyma. He further stated that, although rarely, some monocots also have collenchymateous transmitting tissue. As noted by Vasil and Johri (1964), Guéguen and others apparently misinterpreted dissociated transmitting tissue with its gelatinized middle lamella as collenchyma.

Joshi (1934) used the term stylar canal to "...include both hollow and solid types and the whole pollen-tube conducting tissue of the style and its extensions into the ovary wall which sometimes may reach the very base of the carpel." He concluded that solid styles are primitive and that the stylar canal has been derived from, and represents, modified ventral bundles of the carpels.

Joshi's interpretations were shown to be incorrect by Arber (1937) and many others since that era. In reference to transmitting tissue, Arber wrote, "...since this tissue proves to be a secretory epidermis (and sometimes subepidermal layers) belonging to the inner faces of the carpellary margins, its existence is in no way incompatible with the Candollean view of the carpel, for localized secretory activity of the epidermis is a familiar feature of leaf structures."

This terminates the introductory remarks for the carpel, stigma, and style portion of this review. General remarks concerning ovules will precede the section on ovules. A comprehensive review of every paper containing information on these subjects (and those yet to come) is way beyond the scope of this dissertation. The papers which I have
included were chosen for one or more of several reasons: (1) importance to the subject matter as determined by reputation (good or bad) and frequency of citation; (2) direct pertinence to the subject; or (3) they came to my attention by hook or by crook and I found them interesting. Many papers with a promising title were perused and reshelved immediately because they proved to be either: (1) irrelevant; (2) redundant of other superior papers; or (3) not worth the powder to blow them up.

Because of the broad scope of this paper, I have chosen to break it down into more convenient morphological, anatomical, and physiological entities for the rest of the review. There are six major sections: carpels, stigma, style, ovules, pollen tube growth, and floral responses to pollination.

As I write and as you read, we should both try to keep in mind the introductory material so as to maintain a picture of where the part fits into the whole while investigating that part. It is my hope that by so doing we will have a good framework in which to integrate the results of the current research and then build the concept of the flower as a biologically functional system in the discussion section.

Carpels

Epidermis

The external carpellary epidermis generally consists of a single layer of cells. These cells vary in shape from elongate or palisade--like in Polygonum (Neubauer, 1971)--to isodiametric in Lychnis (Oostenink, 1976), while palm epidermal cells may be either cuboidal or elongate depending on the species (Uhl and Moore, 1971). The external carpellary
epidermis in some taxa, e.g., *Arachis*, may become sclerified (Halliburton et al., 1975). The palm epidermis shows variance also in the thickness of its cuticular layer (Uhl and Moore, 1971), as do other taxa, e.g., *Polygonum* (Neubauer, 1971) and *Averrhoa* (Dave et al., 1975) with thin cuticles, and *Capsicum* (Munting, 1974) and *Lychnis* (Oostenink, 1976) with thick cuticles.

**Cuticle** Brongniart (1834) described the cuticle as the outer, cellulose-free covering of leaves, and von Mohl (1845) observed the continuation of this surface cuticle into the respiratory chambers of stomata and the immediately adjacent intercellular spaces of the mesophyll. In 1871, De Bary published an excellent discussion with a supporting series of drawings of wax on leaves. He distinguished granular, columnar, and platelike structures in the wax layer and postulated the extrusion of wax through the cuticle. According to Van Wisselingh (1895), the true cuticle is formed by the oxidation of the oily materials which ordinarily permeate the walls of living cells. He assumed the oils to be products of cell metabolism.

The idea that wax may be extruded onto the surface through pores was postulated by Dous (1927). Weber (1942) failed to find pores in the cuticle and, similar to Van Wisselingh (1895), concluded that the wax is secreted in a liquid form which infiltrates through the epidermal wall and cuticle, later coagulating into the structures described by De Bary (1871). Kreger (1948) made extensive x-ray and limited EM studies of wax, and discussed the possible methods of wax crystal formation. Scott et al. (1958) claimed the presence of plasmodesmata in the outer wall of onion epidermis, with transcurrent pores or sievelike areas in line with
the plasmodesmata. They did not use sectioned material, however, and they failed to actually demonstrate the presence of the external plasmodesmata.

Von Mohl's (1845) observations on the continuation of the cuticle into stomatal chambers have been supported and expanded. Arzt (1933) and Scott and Lewis (1953) demonstrated the presence of a cuticle over the surface of mesophyll cells where these cells are exposed to intercellular air passages, and Lyshede (1977) has demonstrated the presence of wax in stomatal cavities of *Anabasis* and *Calligonum* with a scanning electron microscope. Scott (1950) postulated that a layer analogous to the cuticle, composed of suberinlike material, is deposited throughout the whole system of intercellular spaces of the stem, root, flower, and fruit, as well as the leaves. Scott stated that the chemical precursors for this material are laid down in very young cells as monomolecular layers on the inner face of the cell wall by plasmalemma activity. These stuffs then pass through the cell wall and eventually form a film on contact with air. The degree of internal suberization varies with age and type of tissue, and with the habitat of the plant in question.

Mueller, Carr, and Loomis (1954), working with replicas, vice sectioned material, showed that species which develop surface accumulations of wax show small pits or craters in the cuticle whereas those plants without surface wax do not. They further showed that the pits exhibit patterned distributions and that they develop continuously as the leaf develops. In addition, they demonstrated that as wax weathers away with age, the cuticle undergoes chemical changes to compensate for the loss of the wax. Mueller et al. concluded that the cuticle is the real
protecting layer and that the surface wax probably has no important role in transpiration. They found no evidence for the secretion of waxes in liquid form and felt that the best hypothesis is that wax is extruded in a softened form under some pressure, and that in some plants the wax may errupt through the cuticle. Fahn et al. (1974) postulated that the epicuticular wax on the juice vesicles of Citrus fruits serves as an adhesive to hold adjacent vesicles together, and Shiraishi et al. (1974) use the quantity and structure of the epicuticular wax of mandarin orange floral parts as a clue to productivity levels for the ensuing crop.

Schieferstein and Loomis (1959) showed that wax, and possibly cutin also, may accumulate beneath the cuticle and may more or less completely impregnate the entire outer epidermal wall. They concluded that "...wax is extruded through the young, fragile cuticle, and that the normal thickening of this layer both stops wax extrusion and closes the openings through which it was extruded. The presence of wax pores extending to the surface of the leaf, as assumed by many, thus appears to be precluded."

The argument as to the mode of wax extrusion was renewed by Hall (1967a,b) with the suggestion that special canals in the wall and cuticle, ectodesmata, are involved. Martin and Juniper (1970) did not find any such canals, and Schönherr and Bukovac (1970) concluded that ectodesmata are not specific cell wall structures, but rather that they are areas of cuticle permeability to polar compounds such as heavy metal salts.

Cuticles form centripetally, the outer portion being laid down first (Fahn, 1974) and are composed of two distinct layers. The cuticle adheres to the cell wall by way of pectins (Walker, 1975c), and, in many plants, there is an additional layer of epicuticular wax deposits on top of the
cuticle proper. The inner, or cuticular layer, is basically the cutin-impregnated outer portion of the epidermal cell wall. The outer layer, or cuticle proper, is a layer of cutin on top of the cell wall (N. R. Lersten, Iowa State University, personal communication, 1977).

In Apium, Eryngium, and Humulus, this outer layer is lamellate with alternating electron dense and electron transparent plates 5-8 nm in thickness. In species of Abutilon and Rumex, the cuticle proper does not exhibit the lamellate structure, but all five genera do have a reticulate component in the inner layer (Chafe and Wardrop, 1973). In Phorium, the reticulate layer thickens with development, and in places it may form papillae (Jarvis and Wardrop, 1974). The reticulate network has been implicated in the secretion of cutin precursors (Chafe and Wardrop, 1973) and the lamellae in the secretion of wax (Hallam, 1970a, b). Walker (1975a, b, d) has shown that cell-to-cell contact during the fusion of free parts, e.g., postgenital carpel fusion, results in the cessation of cuticle deposition. The cuticle already present, however, remains intact within the fused wall.

The surface continuity of the epidermis of many carpels is interrupted by the presence of stomates, e.g., Bakeridesia (Klotz, 1975) and Averrhoa (Dave et al., 1975). In the peanut, lenticels develop on the aerial section of the peg portion of the ovary (Smith, B. W., 1950). Stomates may be found on the style, and as part of both the outer and inner epidermis of the ovarian region (Fischer, 1929). Nonfunctional stomates are reported to be in the inner epidermis of the style of Lilium tigrinum (Vasil and Johri, 1964). Some carpels, e.g., Chelidonium majus (Fischer, 1929) and Butia capitata (Uhl and Moore, 1971), have sunken stomates.
The presence or absence of stomates in grass carpels is uncertain, but for the most part seem to be absent. Although no true stomates are present in the pericarp of barley (Bawtree and Gordon, 1965; Pomeranz and Sachs, 1972a), there are stomatellike cells (Pomeranz and Sachs, 1972a). Other anatomical studies and reviews of grass caryopses make no mention of stomates (Alexandrov and Alexandrova, 1943; Pomeranz and Sachs, 1972b; Rost, 1973; Rost and Lersten, 1973).

**Stomates**  Stomatal types are classified on the basis of their ontogenetic development, and *Ornithogalum* leaves are reported to have anomocytic stomates (Belyanina and Krylova, 1971). The classification scheme has recently undergone a revision by Fryns-Claessens and Van Cotthem (1973). According to them, aperigenous stomates develop by the guard cell mother cell dividing once to form the two guard cells. The surrounding epidermal cells are neighboring cells independently derived from the protoderm. The mature stomata are devoid of subsidiary cells and belong to the anomocytic type. Thus, the stomates of *Ornithogalum* are classified as the aperigenous anomocytic type.

Tomlinson (1974) has attempted to devise a system for use of the developmental pattern of the stomatal complex as a taxonomic character in monocots. According to Tomlinson, the developmental pattern characteristic of many members of the Liliflorae, e.g., Liliaceae, is that in which the neighboring cells are without derivatives. In this type, subsequent to development of the meristemoid, there are no divisions in the neighboring cells which directly become contact cells.

A recent study of the epidermis in tobacco leaves (Pankucsi, 1976) has shown that there is a variation in the number of stomates per unit
area depending on the time of planting. Plants from seeds sown in the earliest of the spring plantings had the greatest number of stomates. Since *Ornithogalum* flowers year-round in the greenhouse, it would be interesting to make a comparative seasonal study of stomatal frequency in the carpels and see if there is a difference. If a difference does exist, it would be of further interest to determine if there is any correlation between carpellary stomatal frequency and characteristics of the seeds developed within the particular ovaries such as nutrient quality and quantity, seed abortion, and ability to germinate.

**Cell inclusions** As a rule, epidermal cells are highly vacuolate, but numerous mitochondria, ER cisternae, spherosomes, and dictyosomes are commonly present in the cytoplasm (Fahn, 1974). In *Lychnis*, however, the carpellary epidermal cells are filled with dense cytoplasm having small, scattered vacuoles (Oostenink, 1976). In *Catharanthus*, they are less vacuolate than the cortical cells (Walker, 1975c), and the dictyosome and ER vesicle populations climax during the period when carpel fusion is occurring (Walker, 1975d). See also Kristen (1973) for ultrastructural information on epidermal cells.

Epidermal cells in general have been reported to normally contain a wide variety of stuffs including bacterial aggregations and viruses (Lewis and Crotty, 1976, 1977), mineral crystals (Zindler-Frank, 1976), protein crystals, starch grains, anthocyanins and rhodoxanthins, flavones, phlobaphenes and other tannins, silica bodies, various acids, alkaloids, organic compounds, and salts, as well as a variety of fat bodies (Linsbauer, 1930).

**Lipotubuloids and spherosomes** One of the types of fat body inclusions, of pertinent interest to *Ornithogalum* carpels, has been variously
termed elaioplast, oléoplast, oléosome, olkörper, oil body, Zellenbläschen, Zellenkörper, fatty body, elaiosferer, oeloplastid, oléoleucite, élément oléifere, and système oléifere. Generally the term olkörper has been restricted to liverworts while elaioplast has been restricted to monocots, but as noted by Faull (1935) the tendency since the early 1900's has been to consider these as essentially the same.

According to Faull (1935), "Elaioplasts are a heterogeneous group of intracellular bodies presenting the characteristics of fatty substances to a marked degree but not recognizable as ordinary types of plastids, chondriosomes or vacuoles. ...They have been variously described as aggregations of lipoid globules, as modified or unusual types of plastids or vacuoles, as nuclear derivatives, as aggregations of mitochondria-like bodies or as independent structures. They have been linked with various physiological processes such as assimilation, excretion or degeneration."

Recent observations with the electron microscope have shown that Faull's (1935) definition falls short of the mark. A series of papers by Kwiatkowska (1966, 1971a,b, 1972a,b,c, 1973a,b) has settled many of the questions unsolved by earlier workers, and has led her to rename and redefine this structure. According to Kwiatkowska (1971a), they "...consist of agglomerations of osmiophilic granules within the cytoplasm, caught in a network of, and interconnected by, a system of microtubules. These structures have been named lipotubuloids. They translocate in the cell by way of a progressive rotatory motion. ER, ribosomes, and scarce mitochondria and dictyosomes occur within the lipotubuloids."

Kwiatkowska supports her name change from elaioplast to lipotubuloid on the basis that the term elaioplast connotes the entity as being a
plastid, when in fact it is not, nor is it even bordered by any kind of finite structure as a membrane. She defines lipotubuloids as "...osmophilic granule aggregations webbed and joined by a system of microtubules formed within the groundplasm and translocating in it as a whole body." This is the term and the definition that will be used in this dissertation.

The earliest references to lipotubuloids are those of Mirbel (1835) and Gottsche (1843), but they are lacking in completeness. The first detailed descriptions were by Pfeffer (1874), Wakker (1888), and Lidforss (1893). These three papers provide a description of the three main types of mature lipotubuloids. Pfeffer (1874) described those characteristic of liverworts; Wakker (1888) described those of most tissues of flowering plants, particularly Orchidaceae, Liliaceae, Amaryllidaceae, Iridaceae, and Malvaceae; and Lidforss (1893) described those of angiosperm leaves. Beer (1909) added Compositae to Wakker's list. In addition to these main types, there is a smattering of other descriptive types, including some from fungi and algae (Faull, 1935).

As noted by both Faull (1935) and Kwiatkowska (1966), the lipotubuloids of Vanilla, as described by Wakker (1888), have served as the prototype for lipotubuloid investigations in other flowering plants. Wakker described a stroma with embedded oil globules and showed that, although the oil bodies may project into the vacuole, they are cytoplasmic structures and do not exist in the vacuole as thought by Pfeffer (1874). Pfeffer described them as aggregations of homogeneous oil globules in the vacuole, and Beer (1909) described them as an aggregation of smaller bodies, each composed of a stroma with included oil globules. Pfeffer (1874) inferred the presence of an enveloping membrane around the
lipotubuloids found in liverworts, as did Raciborski (1893a) for those found in monocots.

The formation of lipotubuloids was considered to be a process involving the aggregation of small oil drops by Pfeffer (1874) and by Lidforss (1893). Wakker (1888), however, felt that a stroma develops first and then later the refractive oil drops form within the stroma. Raciborski (1893a) believed they formed by the growth of refractive drops with subsequent fusion of the bodies so formed. Beer (1909) described them as forming by the aggregation of degenerating plastids with the subsequent production of oil.

A fragmenting and budding of the bodies when they are past maturity was first described by Raciborski (1893a). Budding occurs by the formation of small bulges along the perimeter of the lipotubuloid. The bulges later detach themselves from the mother lipotubuloid and then remain free in the cell. Raciborski (1895) and Politis (1911) believed budding to be a multiplicative process within a given cell. Tourte (1966) claims that during mitosis the lipotubuloids of Vanilla, Ornithogalum, Haemanthus, and Polyanthus become polarized so that each daughter cell will receive an equal portion.

Kwiatkowska (1966, 1971b) demonstrated that budding is the first step in lipotubuloid degeneration, and that it occurs when the carpels lose their green color. Subsequently, autolytic vacuoles which contain hydrolytic enzymes appear in the aging lipotubuloids, and then the microtubules disappear. The final stage is their dissolution into single lipid granules (Kwiatkowska, 1971b). Whereas in monocots the lipotubuloids generally degenerate after reaching maturity, those in liverworts persist
even until cell death (Faull, 1935).

The rotatory movement of lipotubuloids in monocots was first reported by Zimmermann (1893), and the Brownian type of movement in hepatics by Dombray (1926). Faull (1935) concluded that rotatory movement is not natural, but rather that it occurs as a result of injury and is later followed by cell death. Kwiatkowska (1972b), with the aid of electron microscopy, has shown Faull to be in error. She has demonstrated that lipotubuloids move by two separate forces resulting in a progressive rotatory motion. The progressive motion is dependent on cyclosis and the rotatory motion is autonomous. The diameter of microtubules associated with the lipotubuloids changes with a change in the speed of rotation, and Kwiatkowska correlates microtubule size change with microtubule contraction and the subsequent rotatory motive force.

Raciborski (1893a) considered lipotubuloids to be an independent system free from other cytoplasmic entities within the cell, and that they arise de novo, while Wakker (1888) regarded them as special plastids. Beer (1909), working with *Gaillardia*, believed them to be the product of chloroplast degeneration with a secondary function of coloration. Guillermond (1922) believed them to be "...aggregations of ergastic substances in the form of lipid droplets held together by the force of adhesion."

In liverworts, Dombray (1926) claimed that they arise by way of vacuole transformations and that they have a protective function.

Faull (1935) disagreed with Dombray's idea of a vacuolar origin in the Jungermanniales, and she concluded that the lipotubuloids in *Iris* represent a seasonal phase of ordinary plastids. She said that in *Iris* the lipotubuloids are the resting phase of leucoplasts which persist
through the resting season and then produce starch through the growing season. Faull stated further that lipotubuloids in such organs as leaves, roots, and bulbs or those found widely distributed throughout the plant, as those in Vanilla, are active plastids whereas those in floral organs are probably degenerate plastids.

Faull (1935) thus supports the earlier contentions of Wakker (1888) and Beer (1909), among others, that postulate a relationship with plastids rather than with vacuoles or with the nucleus as did a few early workers. She did not, however, preclude the possibility that some lipotubuloids may be "...more or less fused aggregations of oil globules which bear no relationship to plastids."

In Ornithogalum umbellatum, the first signs of lipotubuloid development appear when the ovary is about one mm long, and development reaches a peak 3-5 days after anthesis. There is generally one per cell, but, upon occasion, more may be found. The lipotubuloids form by small groups of osmiophilic granules merging to form one structure which continues to grow by the formation of one or more granules within it (Kwiatkowska, 1971b). The granules have all the physical characteristics of spherosomes, and some of them have all the hydrolytic enzymes (Kwiatkowska, 1966). Some ER, ribosomes, mitochondria, and dictyosomes lie around and between granules, the latter being composed of unsaturated lipids, neutral lipids, phospholipids, and proteins. Lipotubuloids also contain acid and alkaline phosphatases, nonspecific esterases, and lipases (Kwiatkowska, 1966). Malva and Althaea have lipotubuloids which are chemically similar to those of Ornithogalum (Kwiatkowska, 1972c).
Each *Ornithogalum* granule is bound by a half-unit membrane (Kwiatkowska, 1973a) and is in contact with tens of microtubules. The number of microtubules increases proportionally with lipotubuloid growth. Microtubules are usually grouped in bands in various planes on one granule and one band may adhere to several granules. The microtubule bundles have a tendency to arrange themselves parallel to the lipotubuloid surface, although they run in various planes resembling "...wool strands wound up in a ball." Microtubules which occur around the lipid droplets closely adhere to the droplets (Kwiatkowska, 1971a) and have a layer of polysaccharide material located on their outer surface (Kwiatkowska, 1973b). The microtubules probably function as both a skeleton framework and as an intra-lipotubuloid transport system (Kwiatkowska, 1971b), as well as a locomotive system (Kwiatkowska, 1972b). Lipid bodies also occur in the cytoplasm outside of lipotubuloids, but they do not have any microtubules associated with them (Kwiatkowska, 1971a).

The cytoplasm within young lipotubuloids is characterized by numerous vesicles with smooth membranes which disappear with age. Vesicles with rough membranes appear later and are about the same size as the osmiophilic granules. Some of these vesicles have microtubules surrounding them, and some have microtubules inside the vesicles as well. The latter probably represent granules with their electron-dense contents gone (Kwiatkowska, 1971b). The epidermal cells become vacuolate after the perianth dries up. This is accompanied by the center of the lipotubuloid losing its granules with a pronounced concurrent activity of acid phosphatase and lipase (Kwiatkowska, 1966).
Lipotubuloids have been shown to be the primary site of $^3$H-palmitic acid incorporation within the cell, and thus the site of lipid synthesis. A secondary localization was found in the outer layer of the cell wall just below the cuticle. The lipids synthesized within lipotubuloids were shown to be distributed throughout the cell at a later stage (Kwiatkowska, 1972a).

No unique elements have been found in lipotubuloids, all components being found in normal cytoplasm. Galatis and Apostolakos (1976), however, have shown that the microtubules in the lipotubuloids of Marchantia are different from those in the cytoplasm in that they are not surrounded by a clear halo of cytoplasm as are normal microtubules. Lipotubuloids do, however, have the specific feature of many microtubules in association with the lipid granules, owing to which the lipotubuloids exist as cytoplasmic entities (Kwiatkowska, 1971a). Faull (1935) concluded that lipotubuloids are probably involved in reserve food storage, and Kwiatkowska (1971b) said that they may be somehow involved in the cell cycle, but that their ultimate function is yet to be disclosed.

Wakker (1888) stated that there is no connection between lipotubuloids and calcium oxalate crystals found in the same plant, but Wóycicki (1929) found protein crystals forming in some of the lipotubuloids of Ornithogalum caudatum. According to Wóycicki (1929), lipotubuloids occur only in bracts, perianth leaves, and the outer carpel wall of O. caudatum. They consist of an oily central region surrounded by a protein cortex that is in turn bordered by a granular zone. The protein material in the bracts becomes crystalloid and then breaks up into smaller crystals. The lipotubuloids were noted to disappear with age (Wóycicki, 1929).
There seems to be some disquiet in the literature concerning the common acceptance of a definition and ontogenetic history of the entities termed spherosomes and oil bodies, and frequently the terms are used as synonyms. According to Frey-Wyssling et al. (1963) and Frey-Wyssling and Mühlethaler (1965), ER forms spherosomes which in turn form oil bodies, and a concomitant decrease in electron density accompanies oil body maturation. Both spherosomes and oil bodies are bound by a tripartate unit membrane.

Schwarzenbach (1971), working with castor bean endosperm, supports Frey-Wyssling and Mühlethaler's (1965) general postulate that spherosomes originate from ER. In addition, Schwarzenbach noted that the bounding unit membrane undergoes differentiation so that the mature oil body is surrounded by only the outer layer of the original unit membrane. Although no succinct statement is made on the point, Schwarzenbach implies that the oil is synthesized between the layers of the original unit membrane. Going one step further, Kristen (1975) defines the spherosomes in Nomaphila stricta, a liverwort, as lipid bodies without a bounding membrane.

Also working with Ricinus communis endosperm, Harwood et al. (1971) noted particulate, vacuolelike inclusions within the developing oil droplets. Too, they noted that the oil droplets contained the necessary enzymes for triglyceride synthesis and proposed that the particulate, vacuolelike inclusions are the sites of oil synthesis. The oil droplets would thus grow centrifugally from a core of inclusion bodies.

Schneider and Seaman (1977) report that lipid bodies of Fusarium sulphureum are first seen as electron dense areas within dilated ER
cisternae. One or more such cisternae may be formed within a single strand of ER, and, when mature, the intracisternal lipid bodies usually become detached. The lipid bodies are bound by a unit membrane. For a more detailed discussion of spherosome membranes, see Yatsu and Jacks (1972).

That spherosomes do not react positively to stains for neutral fats but rather do give a positive reaction to phospholipid stains was shown by Sorokin (1967). Sorokin thus concluded that spherosomes cannot be direct precursors of neutral fat droplets. Rest and Vaughan (1972) found that, although ER was present at the time oil bodies were forming in Sinapis alba, the oil bodies seemed to arise directly from the cytoplasm and not from the ER.

Smith (1974), with Crambe abyssinica, differentiated between spherosomes and oil bodies. He showed that spherosomes develop from ER while oil bodies form from small areas of electron dense particulate material. These particulate aggregates are probably of a proteinaceous nature and may or may not be associated with ER. The amount of particulate material surrounding the oil body decreases as the oil body increases in size.

Spherosomes have been shown to contain a number of hydrolytic enzymes (Kwiatkowska, 1966; Smith, 1974) and have been implicated as being part of a given cell's lysosomal system (Matile and Spichinger, 1968). In Crambe, vacuoles acquire their acid phosphatase activity from the spherosomes. Once associated with a vacuole, a spherosome forms a continuous, thin layer of acid phosphatase active material closely appressed to the inside of the tonoplast (Smith, 1974). For further information on the lysosomal system and the involvement of spherosomes therein, the reader
is directed to Gahan (1967), Matile and Spichinger (1968), Matile and Moor (1968), Matile (1968, 1975), and Berjak (1972).

Traditionally, carpellary tissue is divided into three zones following fertilization: exocarp, mesocarp, and endocarp. For purposes of this review, however, such zonation will be modified to some extent. The carpel will be considered histologically in the following terms: the external epidermis, covered in the preceding section; the cortex, defined to include all of the tissues between epidermal layers; and the internal epidermis. This modification does cause some overlapping of the classical zones, but it eliminates the necessity for referral to the temporal considerations of zone formation. Where temporal or zonal considerations are of import, however, such will be indicated.

Cortex

Growth Once carpels have been initiated, carpel growth is generally the result of an increase in cell size rather than in cell number (Sinnott, 1939; Riley and Morrow, 1942; Smith, 1950). In Lychnis, however, mitotic figures are frequent in all cell layers of the young ovary wall, but following anthesis there is no further addition of new cells (Oostenink, 1976). Avocados, on the other hand, continue to exhibit some mitotic activity up until fruit maturity (Schroeder, 1953). In cucurbits, the size at which cells divide increases with age, and, in large fruited races, the period of cell division and of cell expansion are both longer than in small fruited races. The larger size of the former is thus due to both more cells and larger cells (Sinnott, 1939).
Fruit growth in *Capsicum* does not take place regularly over the whole length of the fruit. With India ink marks, one can show that there is a growth zone at the base of the fruit (Munting, 1974). A somewhat similar situation occurs in the peanut where an intercalary meristem in the base of the ovary becomes active following syngamy. This meristem produces the so-called peg which causes the seed-bearing portion of the ovary to go underground. The peg is, therefore, an elongated portion of the ovary and is not a gynophore as purported by Charles Darwin and subsequent workers. An interesting further observation on the ovaries of *Arachis* is that they can absorb water and nutrients from the soil once they become subterranean. The ovaries have an absorptive feature capable of sustaining a plant for several weeks following removal of the roots (Smith, 1950).

**Vascular tissue** The carpellary cortex may contain a variety of cell types including parenchyma, chlorenchyma, aerenchyma, collenchyma, and sclerenchyma as well as several types of idioblasts. The vascular tissue which feeds the carpels and joins with the traces which supply the ovules is found here also. Carpel procambialization proceeds acropetally (Esau, 1942; Boke, 1949; Sampson and Kaplan, 1970). The transition from initial to procambial cell is gradual, and, according to Esau (1942), no clear distinction can be made between the developmental stages.

Carpels most commonly are supplied with one dorsal trace, two ventral traces, and two lateral traces, but there is great variety in number and pattern among different taxa. The palm, *Nypa fruticans*, holds the record for the highest number with 150-200 vascular bundles, and each has a large fibrous bundle cap (Uhl and Moore, 1971). The presence of a bundle
cap is apparently not a common feature of carpellary vasculature. The report of their occurrence by Uhl and Moore is the only reference to this character that I have encountered.

Carpels exhibit variety in their vasculature not only in the number of veins, but in bundle type and other characters as well. For example, sieve tube plastids from shoot and leaf tissue have been shown to be taxonomically unique (Behnke, 1972, 1975; Behnke and Dahlgren, 1976), and this study will show that plastids from floral vascular tissue are of value in this regard as well. Most commonly, carpellary traces are bicollateral, but, in *Papaver somniferum* (Kapoor, 1973), eleven amphicribral bundles form by the fusion of 3-4 collateral bundles each at the base of the ovary. Fusion is such that the xylem is oriented toward the center with phloem to the outside producing the amphicribral pattern. Toward the carpel tip, the amphicribral bundles become reduced to collateral bundles once again. The Psoraleae of the Leguminosae have a unique vascular pattern which results in a discontinuity plate. At the base of the ovary, the three ovarian bundles merge with a horizontal proliferation of tracheary elements. This platelike xylem is not connected with the xylem of the pedicel, hence the name, discontinuity plate (Lersten and Wemple, 1966).

**Specialized cells** Many, if not most, taxa have carpels which contain chlorenchyma, an observation verified quite easily by a visit to any greenhouse. Not quite so readily apparent, but probably of equally widespread occurrence is the presence of sclerenchyma, be it as fibers, sclereids, or sclerified parenchyma. Sclerified cells are very prominent in the carpels of the peanut (Smith, 1950; Halliburton et al., 1975),
some grasses (Narayanaswami, 1953), many legumes (Fahn and Zohary, 1955),
palms (Uhl and Moore, 1971, 1973), *Allenia* (Chenopodiaceae) (Werker and
Many, 1974), pecan (Dozier and Amling, 1974), Cunoniaceae (Dickison,
1975), *Bakeridesia* (Malvaceae) (Klotz, 1975), and *Lychnis* (Caryophyllaceae)
(Oostenink, 1976) among many others.

In the peanut, there are three uniquely shaped sclereids which are
arranged in oblique layers, much like plywood (Halliburton et al., 1975).
Grasses also have unique cells known as cross cells and tube cells.
These cells have thick lignified walls with simple pits. The cross cells
parallel the surface but elongate transversely to the long axis and may
branch. The tube cells also parallel the surface, but they elongate
axially (Rost and Lersten, 1973). In the heterocarpous genus, *Allenia*,
among other characters, the two carpel types differ in the thickness of
individual fibers as well as total thickness of the fiber strata (Werker
and Many, 1974).

Aerenchyma, or aerenchyma-like tissue, has been reported to occur
in the carpels of *Aphanopetalum* (Dickison, 1975) and *Lychnis* (Oostenink,
1976). In *Lychnis*, the intercellular air spaces resemble those of leaf
mesophyll, and, although they are present throughout development, they
disappear at ovary maturity. Some leguminous species have collenchyma
(Fahn and Zohary, 1955), as does *Capsicum annuum* (Munting, 1974). The
degree of collenchyma development in *Capsicum* varies with the particular
variety.

Idioblastic cell types of a wide variety are of common occurrence,
and frequently more than one type is found in the same carpel. One of
the best known examples is the occurrence of astrosclereids in pear
fruits. These stone cells also occur in some species of legumes (Fahn and Zohary, 1955), and brachysclereids occur either solitarily or in clusters in some Palmae (Uhl and Moore, 1971). Silica deposits are also found in palm gynoecia (Uhl and Moore, 1973). Giant cells with thickened walls are found in Capsicum (Munting, 1974), and enlarged mucilage cells containing a "...frothy red-staining substance..." are found in Opocunonia and Cunonia (Dickison, 1975). In Lychnis, carpellary cells fill with many small unidentified granules following fertilization (Oostenink, 1976), and cells containing dense granular material have also been reported in Polygonum (Neubauer, 1971).

Tanniferous idioblasts are very common and are found in a broad gamut of taxa, e.g., Pseudowintera (Sampson and Kaplan, 1970), many palms (Uhl and Moore, 1971, 1973), members of the Cunoniaceae (Dickison, 1975), and Averrhoa (Dave et al., 1975). As postulated by Feeney (1970) and supported by Uhl and Moore (1973), tannins may act as a repellent to browsing by affecting tissue palatability. Uhl and Moore (1973) postulate further that in some cases tannins may serve also as an attractant to pollinators or seed dispersing agents. According to Sampson and Kaplan (1970), tannin deposition proceeds acropetally and marks carpel maturation in Pseudowintera.

Crystalliferous idioblasts Crystalliferous idioblasts occur in the majority of plants and have been reported in all tissues of higher plants, including the embryo sac of Aspidistra elatior (Golaszewska, 1934). The only exception to the preceding statement is their absence in pollen grains (H. T. Horner, Jr., Iowa State University, personal communication, 1977). They are quite prevalent in the floral organs of many taxa, e.g.,
the gynoeia of numerous Dilleniaceae (Dickison, 1968), Liliaceae (Sterling, 1972, 1973a,b,c, 1974a,b, 1975), Palmae (Uhl and Moore, 1973), Malvaceae (Klotz, 1975), Cunoniaceae (Dickison, 1975), and Euphorbiaceae (Bor and Kapil, 1976). Calcium oxalate raphides are the most common type, but other crystal forms occur as well. In the carpels of several members of the Dilleniaceae (Dickison, 1968) and of Capsicum (Munting, 1974), they occur as crystal sand, while the carpels of Bakeridesia (Klotz, 1975) and members of the Cunoniaceae (Dickison, 1975) contain druses. Druses also occur in the subdermal tissue in the obturators of Tieghemopanax (Rao, 1972) and Chrozophora (Bor and Kapil, 1976). Crystals in some genera of the Cunoniaceae are noticeably restricted to the placenta and to cells lining the locules (Dickison, 1975).

Developing crystalliferous idioblasts are detectable at an early stage by their overall large size and the large size of their nucleus and nucleolus in relation to neighboring cells (Horner and Whitmoyer, 1972). In Agave (Wattendorff, 1976), raphide crystal cells develop parallel with the leaf axis, and in Psychotria those crystals in the palisade parenchyma are usually oriented perpendicular to the axis whereas in the spongy parenchyma they are parallel with it (Horner and Whitmoyer, 1972). Crystals in Canavalia leaves are located in the epidermis mostly above and below the veins (Zindler-Frank, 1976). Crystals themselves develop within a matrix or slime which is generally held to be some sort of polysaccharide material. This slime in Agave has a reticulate ultrastructure (Wattendorff, 1976). In several aroid genera, e.g., Colocasia, Alocasia, and Xanthosoma, the polysaccharide material swells to a point sufficient to cause the mother cell to burst and
forcefully release the raphides (Sakai and Hanson, 1974).

In Agave leaves, Wattendorff (1976) reported for the first time the occurrence of six-sided raphide crystals that are surrounded by a laminated sheath. Prior to Wattendorff's observations, only four-sided or H-shaped cross sections had been examined ultrastructurally. The laminated sheath, which is about 100 nm thick and composed of lamellae with periods of 6-9 nm, is also a new observation. No polysaccharides were detected within the sheaths by Wattendorff using the Thiéry reaction. The sheaths have prolongations at the small angles of the hexagon which form curls or loops, and these may enclose some of the slime.

Raphides found in Colocasia, Alocasia, and Xanthosoma have two distinct ends. One end tapers to an elongate point while the other terminates in an abrupt point. The crystals are grooved (H-shaped) and have retorsed barbs (Sakai and Hanson, 1974). The raphides of Agave taper equally to a point at both ends (Wattendorff, 1976). For more information on crystals and crystalliferous idioblasts, the interested reader is directed to Horner and Whitmoyer (1972), Zindler-Frank (1976), and Wattendorff (1976) and the literature cited therein.

Internal epidermis

The inner epidermis of the carpel forms the layer which lines the locules. In young gynoecia it is generally uniseriate, but following anthesis it may proliferate to form several layers. In Arachis (Smith, 1950), the inner epidermis becomes multiseriate, forms a spongy parenchyma which serves for temporary storage, and then dries into the white papery layer found inside mature peanut shells. Inner epidermal cells
commonly may become secretory, e.g., some Dilleniaceae (Dickison, 1968), or they may develop thickened walls as in some berry fruits (Kraus, 1949), some legumes (Fahn and Zohary, 1955), and some members of the Cunoniaceae (Dickison, 1975), to mention only a few. In still other cases, the inner epidermis may degenerate at an early stage and disappear completely well before carpel maturation or dehiscence, e.g., Triticum (Alexandrov and Alexandrova, 1943) and Polygonum (Neubauer, 1971).

**Dehiscence**

Dehiscence of carpels in those legumes that have a dehiscent habit is due to differences in the orientation of the axis of shrinkage of the cell walls (Fahn and Zohary, 1955). In the Iphigenieae (Liliaceae), dehiscence is loculicidal (Sterling, 1974b). Referring to loculicidal dehiscence, Sterling states, "This type of dehiscence is undoubtedly a function of the strength of the relatively solid septa (the phylogenetic fusion product of adjacent carpellary walls), which are apparently less likely to be regions of weakness than the dorsal wall in the mature capsule." Septicidal dehiscence occurs in many taxa with the line of dehiscence following the sutures between fused carpels as in Bakeridesia (Klotz, 1975) and Lychnis (Oostenink, 1976).

For a review and discussion of some enzyme activities in gynoecial tissues during fruit ripening, see Yamaki and Matsuda (1977).

**Stigma**

**Morphology and anatomy**

Stigmatic morphology includes a broad spectrum of sizes and complexities.
Smaller, simple stigmas are often found in entomophilous taxa while large, elaborate stigmas are frequently seen in anemophilous plants (Eames, 1961). Many primitive taxa do not have a true stigma per se, but instead have a stigmatic crest which consists of a sessile (no style is present) proliferation of papillate cells along the carpel margins as in Drymes (Bailey and Swamy, 1951).

Relatively simple stigmas may be club-shaped as in the peanut (Smith, 1950), but are more commonly globose or capitate structures as in Petunia (Konar and Linskens, 1966b) and Lilium (Rosen and Thomas, 1970). Some, e.g., Petunia (Muszyński et al., 1976), although having a generally globose outline, may have a centrally depressed area as well. Frequently, stigmas are lobed, papillose structures with the number of lobes corresponding to the number of carpels as in the bilobed stigmas of Datura (Satina, 1944) and Nicotiana (Bell and Hicks, 1976). More elaborate stigmas are simply branched, e.g., Asteraceae (Jones, 1976); highly branched, e.g., sweet potato where it consists of two lobes of 50-75 radiating branches each (Martin and Ortiz, 1967); or plumose with filamentous stigma branches, e.g., sea grasses (Ducker and Knox, 1976). The stigma of Vinca rosea forms a "skirt" of secretory cells around its base at anthesis (Boke, 1949).

Stigmal papillae are usually highly vacuolate (Maruyama et al., 1962), but their cytoplasm is generally dense and the cells have a large nucleus (Rosen and Thomas, 1970). Endomitotic divisions in some of the papillae of Spironema fragrans result in a stigma with polyploid cells of 8 and 4 n, as well as normal diploid cells (Tschermak-Woess, 1959). Cytoplasm in stigmal hairs of cotton is reported to degenerate prior to stigmal
receptivity, but the vacuoles remain intact (Jensen and Fisher, 1969). The papillae of lily (Rosen and Thomas, 1970) and alfalfa (Johnson et al., 1975) lack transfer-cell type wall proliferations of the type described by Gunning and Pate (1969), Pate and Gunning (1969), and Gunning et al. (1970), although other gynoecial tissues have them. Papillae from the stigmas of Narcissus (Chen, 1971) and Brassica (Roggen, 1972) are covered by a prominent cuticle, but those of sweet potato are not (Martin and Ortiz, 1967). Roggen (1972) believes that the wax layer is the incompatibility barrier by its allowing or not allowing pollen grains to adhere to the papillae. Kato and Watanabe (1957) describe a stigma response for some grasses in which papillae collapse and stain more intensely after pollen lands on them.

The upper portion of the stigma, other than the papillae, generally consists of thin-walled parenchyma (Vasil and Johri, 1964; Konar and Linskens, 1966b; Dumas, 1974c). Starch is stored here in some species, e.g., cotton (Gore, 1932; Jensen and Fisher, 1969), and in sweet potato (Martin and Ortiz, 1967) it is arranged in gradually narrowing columns which conduct the pollen tubes from stigma to style. The stigma of Petunia, however, is more complex, and the entire stigmatic region is chlorenchymatous and has both a secretory and a storage zone. Cells of the stigma become filled with starch and oil at an early stage, and the secretory zone forms schizogeneous cavities which fill with the secretory exudate that is released at a later stage (Konar and Linskens, 1966b).
Vascular tissue

In those plants where the stigma is simple and not well-defined, the vascular tissue coming up from the style tends to dissipate distally. On the other hand, in those plants with more elaborate stigmas, the vascular tissue tends to branch peripherally into many delicate strands (Eames, 1961). In addition to the usual conducting elements, the vasculature of some Ericaceae has storage trachieds associated with it in the stigma and upper style (Pohl, 1934).

Style

Anatomy and development

All structures between the stigma and ovary, regardless of number, are ordinarily considered to be stylar in nature (Wilson and Just, 1939). Thus, for example, all of the isolated canals in Citrus which are bound by the morphologically outer surface of the carpel (Guédès, 1973) are part of a single style according to the tenet of Wilson and Just (1939). Stylar number, morphology, and size vary greatly among taxa, and in some instances the latter character may vary within a given species. Distyly, with pin and thrum flowers, is not too uncommon a feature, but tristyly, the occurrence of three different stylar lengths within a given species, is limited to members of only three families: Pontederiaceae, Lythraceae, and Oxalidaceae (Barrett, 1977).

With its two 90° turns and considerably thickened region between turns, the style of the peanut (Smith, 1950) exhibits one of the more interesting variances in stylar morphology. Martin and Ortiz (1967) postulate that in many instances stylar morphology is related to mode
of pollination, and Whitehouse (1950) suggested that long, narrow styles serve as a sieve where incompatible tubes, through inhibition, are screened from compatible tubes. In Solanum mammosum (Martin, 1972), a unique form of female sterility occurs. In the second and all subsequent flowers of a raceme, the style is drastically reduced in length and is nonfunctional. Martin speculated that shortness and sterility are the result of a genetically controlled hormone deficiency.

Stylar development in Vinca (Boke, 1949) and Petunia results from the activity of an intercalary meristem at the base of the style. The meristem of Petunia is active only during the early growth period, and, after the style attains a length of approximately 15 mm (half-mature length), there are no more cell divisions (Linskens, 1974b). In contrast, growth in Datura occurs continually at the base of the style. Growth in stylar length is primarily by cell elongation and in girth by cell multiplication (Satina, 1944). Stigmas and styles of buffalo grass and of corn continue to elongate until they either are pollinated or attain their maximum extension. Buffalo grass styles may reach a length of 1-1½ inches and those of corn may grow to more than 12 inches (Jones and Newell, 1948). In palms where the overall pattern of gynoecial maturation is basipetal, the stigma and upper style are more mature at anthesis than the ovarian region (Uhl and Moore, 1971).

Development of the Petunia style has been shown to occur in three phases: 1) division of cells in the conducting tissue; 2) dependence upon an inductor secreted by the pollen sacs; and 3) dependence upon development of the vegetative part of the flower (Linskens, 1974a). It is interesting to note that castration in Petunia earlier than
100 hrs after the start of stylar elongation up to 4 mm prevents further growth of the style. Castration after 100 hrs, however, allows normal stylar development to occur. Of further interest is the fact that the effect of the removal of flower parts cannot be replaced by the exogeneous application of various types of growth hormones. It seems, then, that an influence from the anthers via the filaments exerts control over development of the corolla and style (Linskens, 1974b).

Vascular tissue

Probably the most common condition for stylar vascularization is continuation of only the carpellary dorsal trace into the style. Tobacco (Bell and Hicks, 1976) and members of the Iphigenieae (Liliaceae; Sterling, 1974b) are examples of this condition. Some members of the Palmae (Uhl and Moore, 1971) and Cunoniacae (Dickison, 1975) provide examples of another condition in which the ventrals also extend into the style. A somewhat unusual situation is found in *Bakeridesia* where the styles contain two collateral bundles, each derived by fusion of a dorsal bundle with a ventral bundle (Klotz, 1975). For a more detailed account of stylar vascularization, refer to Hunt (1937) and the literature cited therein.

 Transmitting Tissue

Stylar

As noted previously, transmitting tissue is the tissue through or upon which pollen tubes grow on their way to effect fertilization. This tissue occurs in varying degrees of development between various taxa,
as well as between different regions of a given gynoeclium. In that the tissue extends from stigma to ovary base (Hedwig, 1793), it may be well developed in the style but not so well-developed in the ovary, or vice versa. Those plants with styles classified as solid according to Hanf's (1935) scheme demonstrate the greatest degree of proliferation within the style, while those with hollow styles show the least.

Transmitting tissue cells of Daucus, derived only from the epidermis, are very rich in cytoplasm and stain intensely. They divide frequently so that proliferation is a result of an increase in both cell number and size. Proliferation begins at the top of the style at the same time the stigma differentiates and proceeds basipetally. The stylar canal is much broader at its base than at its tip, and consequently the proximal end does not fill completely with transmitting tissue. Following Hanf's classification, the style of Daucus is thus the half-closed type (Satina, 1944).

The transmitting tissue in tobacco is chlorophyllous, but the chloroplasts have poorly developed thylakoids. Cells of this tissue also contain amyloplasts (Bell and Hicks, 1976), as they do in cotton (Gore, 1932; Jensen and Fisher, 1969), Lycopersicon (Cresti et al., 1976), and many others (Vasil and Johri, 1964). Transmitting tissue cells of cotton (Jensen and Fisher, 1969) have thick lateral walls consisting of four layers while in tobacco they have a thin primary wall, but the cells are separated by "massive extracellular deposits" (Bell and Hicks, 1976). Plasmodesmata are generally present in all walls of young transmitting tissue cells, but with age they disappear from the lateral walls (Sassen, 1974; Cresti et al., 1976; Bell and Hicks, 1976). A noted exception to
this generality is Capsella where plasmodesmata are reported to persist in the lateral walls (Sassen, 1974). The end walls are generally thin and retain their plasmodesmata to maintain cytoplasmic channels of communication along vertical files of cells (Cresti et al., 1976; Bell and Hicks, 1976).

Transmitting tissue cells of Muscari comosum (Wunderlich, 1937) among others (Behrens, 1895) contain raphide crystals while those of cotton are reported to contain druse crystals (Jensen and Fisher, 1969). In the transmitting tissue of tobacco, Bell and Hicks (1976) claim to have discovered a new type of microbody, i.e., one which contains crystalline material and is surrounded by a double membrane. My careful examination of their micrographs revealed, however, that the double membrane is misidentified and that the second membrane is actually a segment of ER lying very close to the microbody.

It was formerly believed by many investigators that cells of the solid type stylar transmitting tissue have collenchymatous wall thickenings. The thickenings were thought to consist of pectins, hemicellulose and cellulose. Schoch-Bodmer and Huber (1947) reported that pollen tubes dissolve these thickenings while growing through the middle lamella in the styles of Lythrum salicaria. EM studies of styles of Petunia (Pluijm and Linskens, 1966; Kroh and van Bakel, 1973; Sassen, 1974), Diplotaxis (Kroh and Munting, 1967), cotton (Jensen and Fisher, 1969), Capsella, Lythrum, Vitis, and Tradescantia (Sassen, 1974) have verified that the transmitting tissue cells are surrounded by pectin which is digested by growing pollen tubes. Sassen (1974) also feels that the intercellular substance is not middle lamella and is more complex than simple pectin.
He states, in addition, that the transmitting tissue's secretory product is comparable with the substance filling the canals in hollow-styled species. None of the EM studies, however, have shown any collenchyma in the transmitting tissue.

The hollow style of Narcissus has a triradiate canal lined by a single layer of transmitting tissue (Chen, 1971). Cells of this layer in palms vary in length and width (Uhl and Moore, 1971), while in Lilium tigrinum they divide actively and become papillate in an acropetal sequence (Vasil and Johri, 1964). Other Liliaceae (Wunderlich, 1937) have hairlike protrusions in the canal, e.g., Muscarl and Puschkinia. The longest hairs are in P. scilloides, and in M. racemosum they line the whole canal while in M. comosum they are found only in the center. Styles of Lilium (2 spp.), Tulipa, Polygonatum, Convallaria, and Ophio-pogon produce hairs also (Wunderlich, 1937).

Cells lining the canal in many species contain starch (Green, 1894; Vasil and Johri, 1964; Vasil'ev, 1970; Rosen and Thomas, 1970) and some have lipid deposits as well (Vasil'ev, 1970). In Lilium regale and L. davidii, the number of lomasomes decreases and the number of dictyosome vesicles increases while the hyaloplasm becomes more dense as the flowers approach anthesis. Following anthesis, the stylar canal cells become papillose, accumulate starch, and the outer wall thickens to form a Wandlabrinthe in the apical portion of each cell. The number of dictyosomes and mitochondria continues to increase, but SER becomes the dominant organelle. ER surrounds the plastids and the cells resemble terpenoidogenic plant cells and steroidogenic animal cells (Vasil'ev, 1970).
Similar conditions occur in other lilies (Rosen and Thomas, 1970; Dashek et al., 1971), and Rosen and Thomas (1970) have termed the Wandlabrinthe area of the transmitting tissue the secretion zone. Maximum development of this area occurs three days after anthesis (Rosen and Thomas, 1970). Dashek et al. (1971) determined that the outer, fibrillar wall layer contains protein, pectin, and cellulose, while internal to this is a granular-fibrillar layer with outer and inner regions that are cytochemically distinguishable. The granular portion is pectin that is not esterified with methyl groups but may be complexed with protein. This layer also has what they call osmiophilic islands. Alfalfa (Johnson et al., 1975) has been shown to have a Wandlabrinthe region on the centripetal wall of its canal cells also.

According to Vasil'ev (1970), the main function of the transmitting tissue is to secrete mucilage into the stylar canal and the locules. The developmental stage at which secretion occurs varies among taxa, but there is no correlation between stylar type and time of secretion. The most common condition seems to be for secretory activity to occur just prior to anthesis (Sassen, 1974). In Lilium (Rosen and Thomas, 1970; Dashek et al., 1971) some secretory material is trapped between the wall and cuticle prior to anthesis, but expulsion into the canal is not until after bud-burst. Epidermal cells of the stylar canal of Lilium tigrinum fail to secrete any mucilage at all and the style is devoid of it (Vasil and Johri, 1964).

The material secreted by stylar transmitting tissue seems to be basically similar in all stylar types (Sassen, 1974). It is generally a viscous fluid consisting of polysaccharides and proteins, but in
**Lycopersicon** the protein moiety is not incorporated into the exudate until after an extensive proliferation of RER and polysomes in the transmitting tissue cells (Cresti et al., 1976). Exudate production is related to ER in *Petunia* also (Kroh, 1967). The polysaccharides are mainly pectins (Kroh, 1973; Sassen, 1974), and in *Lilium* (Rosen and Thomas, 1970) the pectin precursors are concentrated in the Wandelbrinthe region of the canal cells prior to their secretion. Test results for lipids have always been negative in tomato styles (Cresti et al., 1976), but resins do occur in the stylar tissues of cotton (Gore, 1932). In addition to the aforementioned compounds, Vasil'ev (1970) postulates that stylar transmitting tissue in lily secretes terpenoid sex hormones as well.

**Ovarian**

Terminology for that portion of the transmitting tissue which occurs in the ovary proper is somewhat vague and use of the various terms is inconsistent among authors. The term, obturator, is frequently applied, but there is no concise definition for it, nor is there complete agreement as to the tissue from which it arises. Obturators have been proposed to originate from: the funiculus (Capus, 1878; Juel, 1918; Rao, 1972; Tsai et al., 1973), the placenta (Dalmer, 1880; Lister, 1883; Lloyd, 1899; Houk, 1938; Landes, 1946; Sterling, 1964; Guédès, 1966); and the aril (Rao, 1959) among other tissues, e.g., ovule integuments (Fagerlind, 1944; Eames, 1961; Davis, 1966).

Extant definitions are, at best, general and unclear. According to Eames (1961), "Enlargements of the carpel wall adjacent to the ovule, of
the chalazal region, and of the top of the outer integument that overlap and block, or appear to block, the micropyle to some extent are called obturators. The term is best restricted to protuberances of the carpel."

Davis (1966) is even more vague in her definition: "Any structure which appears to be associated with directing the growing pollen tube toward the micropyle is referred to as an obturator...." In yet another interpretation, Sterling (1964) regards the obturator as "...an internal, placental continuation of the stigmatic surface."

Lloyd (1899) describes a tissue similar to an obturator in several members of the Rubiaceae. He calls this tissue a strophiole, and in reference to it he says, "They are...strictly speaking, cells of a conductive tissue as is shown by the behavior of the pollen tube towards it. ...the strophiole lies against the ovules so as completely to close the micropyle. It will be seen that there is thus formed a collar of columnar epidermis which is continuous around both sides of the funicule and, as will be shown, completes the path of the conductive tissue from the style to the micropyle." In some species the strophiole is nonsecretory as in Diodia teres and members of the Genus Richardsonia, but in others, e.g., D. virginiana, they are glandular. For D. virginiana, Lloyd states, "...the strophiole is glandular and at the time of pollination secretes a layer such that there lies on the surface of the conductive tissue a layer of mucilage of a thickness of nearly half the depth of the cells." Lloyd describes also the occurrence of an obturator in some of the same species in which strophioles are found.

Obturators occur in many taxa, but are especially common in members of the Euphorbiaceae (Maheshwari, 1945), Rosaceae (Eames, 1961), and in
some Liliaceae (Sterling, 1973a, 1974a,b, 1975). They vary greatly among taxa in their anatomy, morphology, and degree of development. In cotton (Gore, 1932), it is described as "...a glandular layer which may secrete a nutrient fluid and thus facilitate the passage of the pollen tube to the micropyle." Gore (1932) implies that the tissue is composed of simple papillate cells. Obturator cells are most commonly papillate, but they may become long and hairlike or even multicellular and filamentous with branching as in *Arum maculatum* and *Lychnis vespertina* (Dalmer, 1880). Obturators protrude into and completely fill the micropyle of ovules of *Daphne*, *Euphorbia*, *Phytolacca*, and *Atherurus* (Dalmer, 1880) as well as other Euphorbiaceae (Schweiger, 1905; Landes, 1946). In what is probably the most prolific condition, the obturator of *Arenaria tenuifolia* completely fills the locule, and in addition to its normal functions may serve secondarily as a paraphysis to keep the ovules damp (Gibbs, 1907).

As previously noted, the observations of Hedwig and of Gleichen in the late 1700's were among the first to attribute to the transmitting tissue a role in the pollination-fertilization process. Amici (1824) was the first, or certainly one of the first, to suggest that this tissue has a nutritive function in regard to pollen tube growth, and the beautifully accurate illustrations of Dalmer (1880) are among the earliest to show pollen tubes actually growing between or atop transmitting tissue cells in the ovary. He shows the obturators of *Ornithogalum nutans* and *O. pyramidale*, with the cuticle separated from the cell wall by slime in *O. pyramidale* as well as in *Mahonia aquifolium*. He also illustrates pollen tubes embedded in the slime on top of the obturator in *Anthericum liliago* and illustrates starch grains in the obturator of *Citrus aurantium*. 
Referring to obturator function in *Stellaria media*, Lister (1883) stated, "In this species, when the flower opens, the dissepiments are detached from the lateral walls of the capsule they have grown broad, and are of very loose tissue; the micropyles of the campylotropous ovules rest on, or are turned towards them, suggesting the idea that these present the course along which the pollen-tubes may be directed to the micropyles." Lyon (1898) suggested an additional function for the obturator of *Euphorbia corollata*, a species in which the nucellar beak grows into the base of the style. He concluded that the obturator guides the directional growth of the nucellar beak "...so that there will be a complete connection between the stigmatic cells and the embryo sac for the passage of the pollen tube."

Many other early workers (Unger, 1855; Molisch, 1893; Miyoshi, 1894; Nawaschin, 1898a,b; Lloyd, 1899; Gibbs, 1907), and a host of more contemporary workers, have supported the contention that the transmitting tissue functions in pollen tube nutrition and helps in directing pollen tube growth. Their ideas on pollen tube growth and the role of the various gynoecial tissues involved therein will be discussed in greater detail in the section on pollen tube growth.

**Plant Glands**

Although the review of plant glands by Lüttge (1971) does not include stigmas, styles, and transmitting tissue by anatomical type, it does discuss them indirectly through consideration of the type of secretory product. Lüttge divides secretory material into five classes: 1) proteolytic enzymes--digestive glands of carnivorous plants, 2) polysaccharides--
slime glands of carnivorous plants and others, 3) sugars—nectaries, 4) inorganic salts—salt glands of halophytes and xerophytes, and 5) products of secondary metabolism—wax, fatty and odorous oils, essential oils, and terpenoid resins. In that stigmatic exudate has been characterized as being primarily lipoidal, the following section will examine the characteristic features of lipid synthesizing cells. This will be followed by a look at glands in general.

Lipid and resin glands

In the resin-producing glands of Cannabis, the resin-producing cellular elements are found almost exclusively in the head of the hairs and in the apical part of the stalk (De Pasquale, 1974). A similar condition occurs in Plumbago (Rachmilevitz and Joel, 1976) where the resin-secreting tissue of the calyx trichomes forms a cap separated from the rest of the trichome by an endodermislike region. Oil secreting hairs of Monarda also have a terminal secretory cell (Heinrich, 1977). The resin-producing cellular elements alluded to by De Pasquale are most likely ER and plastids as these two organelles seem to be almost universally involved in oil secretion. Tubular SER is the dominant organelle in the lipid glands of Ficus, Ledum, and Salvia (Schnepf, 1972). Plastids, closely ensheathed by ER, (usually reported as SER) have been observed in lipid or resin glands of Acer and Pinus (Wooding and Northcote, 1965), Arcticum (Schnepf, 1969a), Calceolaria (Schnepf, 1969b), Solidago (Schnepf, 1969c), Forsythia (Dumas, 1974c,d), Ribes (Tsekos, 1974), Beyeria (Dell and McComb, 1974), Rhus (Fahn and Evert, 1974), Pinus (Fahn and Benayoun, 1976), and tapeta of sorghum, corn, pepper, sunflower,
and Selaginella (H. T. Horner, Jr., Iowa State University, personal communication, 1977). Noted exceptions to the ER ensheathed plastid-lipid secretion association are Viscaria (Tsekos and Schnepf, 1974) and Newcastelia (Dell and McComb, 1975).

Wooding and Northcote (1965) speculate that the sheathing may play an important role in the synthesis of the predominantly terpenoid resin by these cells. The combination of the ER and the plastid membrane is thought by them to form a functional synthetic unit for the production of the terpene moiety of the resin. Both the ER and the plastid members of the association are implicated in the synthetic function by several authors: Tsekos (1974), Fahn and Evert (1974), Dell and McComb (1974), and Fahn and Benayoun (1976). The plastids of Viscaria, which are not ensheathed by ER, are thought to be the site of exudate synthesis in this plant (Tsekos and Schnepf, 1974). ER is postulated to be the excretory product synthesizing organelle in Petunia (Kroh, 1967), Forsythia (Dumas, 1973a) and Citrus oil glands (Thomson et al., 1976). In addition to plastids and ER cisternae, lipophilic substances arise in dictyosome vesicles and mitochondria of the secretory ducts of Rhus (Fahn and Evert, 1974). Dumas (1974c) speculates that the plastid-ER complex provides a site for rapid synthesis or accumulation of secretory materials and a rapid means to transport them within the cell.

**Nectar, mucilage, and salt glands**

In contrast to lipoidal glands, those which secrete nectar or mucilage seem to be dominated by ER and/or dictyosomes (Horner and Lersten, 1968; Schnepf, 1972; Schnepf and Pross, 1976; Rachmilevitz and Fahn, 1973;
Kristen, 1974, 1975; Benner and Schnepf, 1975; Fahn and Rachmilevitz, 1975; Heinrich, 1975). Both the ER and the dictyosomes are implicated in synthesis, intracellular transport, and excretion of the exudative materials in many instances (Horner and Lersten, 1968; Rachmilevitz and Fahn, 1973; Kristen, 1974; Benner and Schnepf, 1975). In Nomaphila (Kristen, 1975) and Lonicera (Fahn and Rachmilevitz, 1975), however, dictyosomes do not seem to participate in secretion so that the ER is probably responsible for the synthesis, transport, and extrusion of the secretory product. With the exception of Spartina, most salt-secreting glands do so by the emiocytotic expulsion of salt-containing "microvacuoles." In Spartina, however, a series of wall protuberances and infoldings of the plasmalemma, which form "partitioning membranes," are probably involved in the excretion of the salt (Levering and Thomson, 1971).

Highly branched wall protuberances also characterize the nectar-secreting cells of Aloe (Heinrich, 1975; Schnepf and Pross, 1976; among many others). See Wergin et al. (1975) for a discussion on the distribution of plasmodesmata and their role in symplastic transport of nectar.

Glandular secretion

Haberlandt (1928) postulated that following synthesis in oil and resin glands, the exudate collects between the cell wall and cuticle before it moves to the cell's exterior. Haberlandt's postulate has been supported by investigations of several systems: Petunia (Kroh, 1967), Arctium (Schnepf, 1969a), Calceolaria (Schnepf, 1969b), Forsythia (Dumas, 1973a), Ribes (Tsekos, 1974), Viscaria (Tsekos and Schnepf, 1974), Newcastalia (Dell and McComb, 1975), and Cannabis (De Pasquale, 1974;
Dayanandan and Kaufman, 1976). Actual extrusion to the outside may be through pores as in *Cannabis* (De Pasquale, 1974) or by rupture of the cuticle as in *Forsythia* (Dumas, 1973a) and *Viscaria* (Tsekos and Schnepf, 1974). In *Rhus*, plasmodesmata have been implicated as a possible pathway for extrusion from secretory cells into the schizogeneously formed secretory ducts (Fahn and Evert, 1974).

In other nonlipid glands, secretion may occur through modified stomata as in the nectar spurs of *Tropaeolum* (Rachmilevitz and Fahn, 1975) and in salt glands through cuticular pores (Arisz et al., 1955; Ziegler and Lüttge, 1966; Levering and Thomson, 1971) or pits (Skelding and Winterbotham, 1939). The secretion of chalk by glands on the leaves of *Plumbago* is through pores also (Sakai, 1974). For a more detailed discussion on the release of secretory products from glands, see Dell and McComb (1975) and the literature cited therein.

**Stigma Receptivity**

The secretion of an exudate onto the stigmatic surface of a wet-type stigma usually denotes that the stigma is physiologically receptive to pollination and the changes initiated thereby. Dry stigmas do not have such an obvious marker of receptivity, and, other than for the presence or absence of germinating pollen on the stigma, one cannot tell by looking at the stigma alone whether or not it is in a receptive state. In many instances, the presence of nectar may provide an externally visible clue of receptivity.
Stages

Working with sugar maple, Gabriel (1966) divided stigma receptivity into several stages based on the morphological and physiological changes of the stigma and pollen: 1) pollen germination, 2) height of receptivity measured by pollen germination and growth, 3) cessation of pollen tube growth—no more pollen tubes are able to penetrate the stigma, 4) cessation of pollen germination, and 5) shriveling of the stigma.

As exemplified by apple (Carlone, 1962), the timing of stigma receptivity is often synchronized with anthesis and embryo sac maturity. Nyéki (1974) demonstrated a similar situation in pear by showing that the highest fruit set occurs when the stigma is most receptive. In Populus, however, the embryo sac is in the four-nucleate stage at stigma receptivity (Fechner, 1972), and, among orchids, the stage of ovule development at the time of stigma receptivity varies greatly with different species (Treub, 1879; Abe, 1972).

Duration

Duration of the receptive period varies among taxa, and in Prunus (Eaton, 1959, 1962) the number of viable egg cells falls sharply the second day after anthesis. By the fourth day eighty percent are degenerate, and by the sixth day all remaining eggs have degenerated. Receptivity in sugar maple lasts approximately 100 hrs (Gabriel, 1966), about five days in 'Tilton' apricot (Facteau and Rowe, 1977), and up to 24 days in corn (Jones and Newell, 1948). Maximum receptivity in buffalo grass occurs during the fifth day after anthesis, and during the eighth day for corn. Buffalo grass is highly receptive up to the thirteenth day,
after which receptivity declines until the twenty-first day when no further seed set occurs. Corn will set seed up to sixty percent at 12 days post-anthesis, but then receptivity falls until the twenty-fourth day (Jones and Newell, 1948).

**Wet and dry stigmas**

During the mid to late 1960's, it became popular to classify stigmas as either wet or dry according to whether or not the stigma is covered by an exudate at the time of receptivity. Examples of wet stigmas are *Petunia* (Konar and Linskens, 1966a), sweet potato (Martin and Ortiz, 1967) and mandarin orange (Shiraishl et al., 1975), while cotton (Jensen and Fisher, 1969) and *Brassica* (Roggen, 1972) provide examples of the dry type. The exudate on wet stigmas has a high surface tension and, therefore, usually appears as small droplets. It is usually sticky, viscous, and highly refractive (Vasil, 1974).

**Stigmatic Exudate**

Disregarding the observations of Gärtner (1844), stigmatic exudates have historically been popularized to be primarily sugar solutions. The stigma was, therefore, regarded as a floral and as an extra-floral nectary (Esau, 1965). Gärtner (1844) examined the stigmas of several taxa and noted the refractive properties of *Nicotiana* exudate along with the slimy nature of that from *Ribes*, *Datura*, and *Physalis*. Gärtner distilled the exudate from *Nicotiana rustica* and *N. paniculata* and obtained a bitter-tasting, shiny, green-yellow material which resembled turpentine.
Characterization

Long after Gärtners work, Baum (1950) characterized the stigmatic exudate of Koelreuteria paniculata as a resin or resinlike substance. Konar and Linskens (1966a) analyzed Petunia exudate and found it to consist of primarily oil with some sugars and amino acids. No proteins were present, but there was a thin film of water beneath the exudate. The oils were pure fat, free from phospholipids, sterols, and free fatty acids, and the principal sugars were sucrose, glucose, fructose, and galactose. Horovitz et al. (1972) found sucrose, glucose, and fructose in the exudate of Yucca, but they only constituted less than two percent of the dry weight. Konar and Linskens (1966a) reported, in addition, that secretion is not stimulated by pollination as exudation occurs in unpollinated flowers to the same extent that it does in pollinated flowers. This fact was noted also by Gärtners in other systems (1844). Tkachenko (1959), however, postulated that in Vitis there may be a threshold stimulus level activated by adequate pollination that turns off the secretory mechanism when a sufficient number of pollen grains have landed on the stigma. Pollination interrupts exudate flow in Lilium also (Labarca and Loewus, 1973).

Working with stigmas of ten species, Martin (1969) performed the most extensive analysis of exudate to date. He found little or no free sugar and determined that the principal components are lipids and phenolics. The phenolics—anthocyanins, flavonoids, and cinnamic acids—occur as glycosides or esters, and the few sugars present are, for the main, tied up in phenolic compounds. Martin (1970a,c), in two other papers, characterized more than 30 lipid and phenolic compounds just from the stigma.
of Zea. Forsythia stigmas and exudate have been examined by Dumas (1973a,b,c,d,e, 1974a,b,c,d) with essentially the same results (but not quality) as did Martin (1969) in regard to the lipid fraction.

Labarca et al. (1970) showed that the exudate of Lilium longiflorum contains both high and low molecular weight compounds, the former being an acidic pectin-protein complex similar to that found in the style. Seagrasses, which remain completely submerged, are apparently a bit different from most angiosperms. They secrete a seawater-insoluble extracellular proteinaceous superficial layer which provides a suitable surface for trapping the water-borne pollen (Ducker and Knox, 1976).

Physiological functions

The stigmatic exudate has a very significant role in the pollination-fertilization process, and each moiety seems to have its own part. Martin and Ruberté (1973) have attributed five functions to the exudate: 1) provides a site for appropriate pollen germination, 2) inhibits foreign pollen germination, 3) nourishes growing pollen tubes, 4) protects the stigma from rain and desiccation, and 5) protects the stigma from insect and fungal attack.

When in the hydrated state, the exudate of Vitis (Tkachenko, 1959) forms large drops which increases the surface area allowing more pollen grains to adhere to the stigma. As the secretion dries down, it concentrates the pollen and brings it into the optimum medium for germination. On amply pollinated stigmas, the drop dries quickly while on poorly pollinated stigmas it remains hydrated for 46-48 hrs. Stigmatic water has been implicated as the source of water for pollen grain hydration prior
to its germination in *Petunia* (Konar and Linskens, 1966a).

Fatty components in general, and fatty acid esters specifically (Konar and Linskens, 1966a), may serve to control the physical conditions for pollen germination by regulating water availability and osmotic conditions. The lipid constituents of the secretion may well correspond to the waxy components of the plant epidermis and serve as a liquid cuticle to prevent water loss via transpiration (Konar and Linskens, 1966a; Martin, 1969, 1970a,b). In addition, the lipid moiety of *Petunia* has been suggested to function as the pollen-trapping agent (Konar and Linskens, 1966a). In some species with dry stigmas, Mattsson et al. (1974) have found what they call a pellicle. This is an external protein coat, over the cuticle, which they suggest to be the agent for trapping and hydrating pollen. They speculate further that it may also be involved in the recognition reaction.

The phenolic compounds probably serve as a source of nutrients for germinating pollen, and probably also play a role in the stimulation and/or inhibition of pollen and pathogen germination (Martin, 1969, 1970a,b). The enzymes diffusing out of pollen and into the exudate probably release free sugars from the phenolic glycosides which then provide proper osmotic conditions for pollen germination and initial pollen tube nutrition (Stanley and Linskens, 1965; Mäkinen and Brewbaker, 1967). The involvement of phenolic compounds with stimulation or inhibition of growth processes is related to their interaction with indole-acetic-acid-oxidase (Van Sumere, 1960; Martin and Ruberté, 1972). Sedgley (1975) has determined, however, that at least the flavonoid moiety in *Brassica oleracea* is not part of the incompatibility reaction for that species.
Michalikova (1970) studied the influence of stigma extracts from several varieties of wheat on the germination of *Ustilago tritici* chlamydospores. He found that extracts from highly susceptible varieties allowed high germination rates while the reverse was true for extracts from more resistant varieties. Martin and Ruberte (1973) tested phenolic extracts from stigmatic exudates for inhibition of fungal spore germination and found that, although there is inhibition, the influence is only slight. They concluded that protection of the stigma from fungal attack is only a secondary function of the exudate, and that rapid germination and growth of pollen on the stigma is the best protection against fungi. Clarke et al. (1977), using pollen and stigma antigens, have recently begun to experiment with the possibility that plant cells, like animal cells, are specified according to their family, species, organ, and tissue by surface determinants. The idea behind these experiments is that recognition genes in self-incompatible plants may specify identical products in stigma and pollen. For more detail on incompatibility, see Linskens (1975) and Heslop-Harrison (1975).

**Stylar Exudate**

Moving down from the stigma into the style, a difference in composition of the exudate encountered is usually noted. The secretory activity of the style can be stimulated by vibrations with cells following the all-or-none law (Büning, 1929), and, in *Lilium regale* and *L. davidii*, it is the dictyosomes that are responsible for most of the secretory activity (Vasil'ev, 1970). Vasil'ev described the stylar exudate in these two species as an amorphous, noncellulosic polysaccharide-containing
mucilage which is produced during the bud stage.

**Characterization**

The intercellular exudate in *Petunia* stylar transmitting tissue is primarily a mixture of acidic carbohydrates (Kroh, 1973; Kroh and Helsper, 1974). In *Lilium longiflorum*, the exudate is ninety-nine percent water and approximately ninety-five percent of the solutes are high molecular weight protein-containing polysaccharides (Rosenfield and Loewus, 1975). Nucleic acid precursors are present also (Campbell and Ascher, 1975), while amino acids have been reported in the stylar exudate of *Pisum* (Wolff, 1975). The mineral content in both pollen and styles varies greatly between species and genotypes as well. Pfahler and Linskens (1974) thus suggest that there is a correlation between maximum pollen germination, pollen fecundity, and the level of various minerals in the style.

**Exudate metabolism by pollen**

That growing pollen tubes absorb, catabolize, and anabolize polysaccharides of the stylar exudate has been demonstrated in a number of instances (Tupy, 1961; Kumar and Hecht, 1970; Rosen, 1971; Kroh et al., 1970, 1971; Kroh, 1973; Kroh and Helsper, 1974; Rosenfield and Loewus, 1975; Mascarenhas, 1975) Mascarenhas (1975) has suggested the following generalized scheme for use of the stylar exudate: "...1) utilization of exudate by growing tubes involves the uptake of polysaccharide fragments from the stylar canal into pollen tube cytoplasm; 2) this is followed by the extensive metabolism to mono- and oligosaccharides of at least a portion of the polysaccharide fragments (the intermediate molecular
weight fraction rich in arabino-galactans). The more acidic, high molecular weight portion is utilized for tube wall formation with little or no breakdown, and 3) these metabolized fragments are then reassembled into the tube wall."

The presence of ribonuclease (RNase) in styles has been demonstrated by Schrauwen and Linskens (1972). They found RNase activity in solid styles to be more than thirty percent greater than in open styles, and that the activity is in or between cells of the transmitting tissue. The absorption of nucleic acid precursors by growing pollen tubes has been shown in *Lilium longiflorum* (Campbell and Ascher, 1975), of amino acids in *Pisum sativum* for both protein synthesis and as a source of nitrogen (Wolff, 1975). Pollen, at least that of *Petunia*, is unable to digest the lipid moiety of the stigmatic exudate and thus derives no nutrition from the fats (Konar and Linskens, 1966a).

As free sugars are not present in significant amounts, the sugar necessary for germination could come from the glycosides of phenolics as suggested previously, and Martin (1970a) summarizes the process of pollen germination with that idea in mind. "The following chain of events may be postulated: pollen lands on the stigmatic surface; enzymes diffuse from the pollen to the stigma (Stanley and Linskens, 1965; Mäkinen and Brewbaker, 1967); phenolic glycosides are hydrolysed; freed sugars diffuse into pollen; respiration of the pollen grain is stimulated (Dickinson, 1965); growth of the pollen tube begins."
Pollen Tube Growth

Once pollen grains have landed on the stigma, they may germinate almost immediately as does *Saccharum* and *Sorghum* (Artschwager and McGuire, 1949), or within 5-10 min as in *Taraxacum* (Poddubnaja-Arnoldi and Dianowa, 1934), *Zea* (Randolph, 1936), and *Hordeum* (Pope, 1937). Some pollen, however, may require hours, e.g., three for *Reseda* (Eigsti, 1937), or even days, e.g., two for *Garrya* (Hallock, 1930), to germinate. The time required for pollen tubes to grow from the stigma to the megagametophyte also varies between taxa, but the time required does not seem to be correlated with the distance over which it must grow. Tubes may complete their growth within 1-2 hrs or within a few days, e.g., *Populus* (Fechner, 1972), or they may take as long as one month to one year, e.g., one month—*Betula alba*, two—*Carpinus betulus*, three—*Alnus glutinosa*, four—*Quercus robur* and *Corylus avellana* (Benson, 1894), and thirteen months for *Quercus velutina* (Conrad, 1900).

Germination

Brink (1924) found that pollen tubes from grains grouped into clumps showed a significant increase in growth over those from single, isolated grains. The growth rate for tubes from single grains and from clustered grains was approximately the same up to a point, and then the clustered tubes grew much faster. He concluded that the growth promoting agent(s) are readily diffusible metabolic products of the pollen tubes and that they are utilized more completely when the tubes are in large populations. From these conclusions, he speculated that the factor(s) are some sort of catalysts.
It has long been known that pollen usually germinates on the stigma and then enters the style. In *Cannabis*, however, pollen germinates along the entire length of the style (Soroka and Zhatov, 1971). Molisch (1893) noted that the pollen tubes of *Narcissus tazetta* are negatively aerotropic, but it was Van Tieghem (1869) who first postulated that a hydrotrropic response is the reason why pollen tubes grow down into the style. The chemotropic response of pollen tubes to the stigma, as demonstrated by Molisch (1893), was regarded by Miyoshi (1894) as being of universal occurrence, and was reconfirmed by Lidforss (1899), Knowlton (1922), and Brink (1924).

**Pathway and method of growth**

As a general rule, pollen tubes grow ectotropically along the surface of the cells lining the canal in hollow-styled species, and endotropically through intercellular spaces of the transmitting tissue in solid-styled species (Vasil and Johri, 1964). It is the rare condition for pollen tubes to actually penetrate stigmatic papillae and grow intracellularly, but it is reported to occur in *Demosonium* (Guéguen, 1901), *Agrostemma* (Hanf, 1935), and in some Malvaceae (Vasil and Johri, 1964). Unger (1855) claimed that the pollen tubes penetrate the cell walls of *Stellaria media* transmitting tissue, but Gibbs (1907) demonstrated that they actually grow along, rather than through, the cell wall in that species.

As noted by Newcombe (1899), enzymes capable of digesting cell wall material were first partially extracted from *Peziza sclerotiorum* by de Bary (1871) and from barley seed by Brown and Morris in 1890. Strausburger (1886) demonstrated that pollen grains could hydrolyze
starch to sugar, and Lloyd (1899) was the first, or certainly one of the first, to postulate that pollen tubes secrete cellulose-dissolving enzymes during their growth. Muir (1942) speculated that growing pollen tubes secrete an enzyme which liberates growth hormones from inactive combinations in the style and ovary. Heinen and Linskens (1961), however, were the first to actually demonstrate enzymatic degradation of stigmatic cutin and that variability exists between plants in their susceptibility to such digestion.

A few years after the work by Heinen and Linskens, Kroh and Munting (1967) showed that once pollen tubes penetrate the stigmal cuticle they grow between the cuticle and the inner part of the cell wall in the cellulose-pectin layer. The tubes grow in this layer to the base of the papillae and then continue growth in the middle lamellae of the stigmatic tissue and the intercellular spaces of the stylar transmitting tissue. Pollen tubes of Petunia (Pluijm and Linskens, 1966) also grow through the middle lamellae of the transmitting tissue, and they have been shown to enzymatically digest a pathway in front of the tube tip. Pollen tubes do not always grow through the middle lamellae of solid styles, however, and in cotton they grow through layer three of the transmitting tissue cells' lateral walls. The lateral wall of these cells is four-layered with the third being especially rich in pectins and proteins (Jensen and Fisher, 1969).

In hollow styles where pollen tubes grow through the mucilage filling the canal, there is no danger of the tubes injuring any stylar cells. With solid styles the possibility does exist and in cotton (Jensen and Fisher, 1969) pollen tubes crush some of the transmitting tissue cell
as then pass by. The crushing results in cell degradation, and undamaged
cells are reported to deposit callose in their pits "...which acts to
prevent transfer of material from these cells to the damaged cells."

Not all solid styles experience injury, however, as is illustrated
by the Rubiaceae. In reference to pollen tubes growing in the styles of
this family, Lloyd (1899) made the following observations. "The pollen
tube does not destroy the cells among which it passes either by chemical
influences, such as digestion, or mechanically. It is true that the cells
are frequently disturbed in position, as plentifully evidenced by the
form of the nuclei when the pollen tube happens to exert pressure on a
cell in such a way as to effect its nucleus (fig. 3) [Lloyd, 1899; his
fig. 3]. Without exception, however, the nuclei and cytoplasm stain
perfectly normally and evenly. The walls of the pollen tubes are to a
considerable degree thicker than those of conducting cells, while the
cytoplasm of the former is more coarsely granular than those of the
latter."

**Chemotropism**

There is some question in the literature on pollen tube growth as to
just how the tubes reach their destination. Brink (1924) summarized
nicely the alternatives. "In seeking an explanation of the direction
of pollen-tube growth in the pistil of the plant, three different possi-
bilities suggest themselves. The tube may be passively guided from the
receptive surface to the micropyle of the ovule by certain anatomical
features of the tissue traversed; it may be oriented in its course by
the diffusion from particular centers of substances having a chemotropic
effect; or, when growing over free surfaces, the pollen tube may arrive at the micropyle purely by chance."

**History and theories** In his physiology textbook, von Sachs (1882) included two chapters on chemotrophic responses of sex cells in lower plants, but the paper by Pfeffer (1884) is considered to be the classical work which initiated the impetus of research in this field (Machlis and Rawitscher-Kunkel, 1963). Pfeffer established that the sperm of liverworts, mosses, clubmosses, horsetails, and ferns react chemotropically to their own archegonia and to some chemicals as well. He did not investigate any angiosperms himself, but he did speculate that pollen tubes also grow toward a chemical stimulant.

Behrens (1875) noted that in transmitting tissue the lateral cell walls separate easily from one another whereas the end walls do not. These facts suggested an axial guidance system by the transmitting tissue for pollen tubes in the style. Dalmer (1880) held that some physical force caused the pollen tube to enter the micropyle, and similarly, Miyoshi (1894) believed pollen tube growth in the style to be purely mechanical. "Im Griffel werden die Pollenschläuche wesentlich mechanisch zum Fruchtknoten gelenkt." Miyoshi showed that the direction of pollen tube growth is not affected by light or gravity, and the results of other experiments by him using excised or injured styles led him to rule out chemotrophic responses. He thus concluded that pollen tubes follow the route of least resistance. Jensen and Fisher (1969) also subscribe to this view, "...the pollen tube appears to be developing down a preexisting path of least mechanical resistance."
After careful investigations of gynoecial anatomy, Capus (1878) concluded that there is no specific anatomical feature which leads pollen tubes to the micropyle. He felt that there is a physiological factor directing tube growth, but he did not know what it was. Strausburger (1878) conducted chemotropic experiments with pollen tubes and ovules of several species, but mainly with Torenia. Using drop cultures, he was unable to demonstrate any specific response of tube growth toward the micropyles. In fact, he found that in most instances when a tube came in contact with an ovule, it would grow over the ovule. Likewise, Correns (1889) was unable to demonstrate any chemotropism with Primula acaulis, a heterostyled species. Correns concluded that chemotropism plays no role in the incompatibility reaction between pin and thrumb flowers. East and Park (1918) were not able to show any definite pollen tube chemotropicism toward a variety of gynoecial tissues either.

Molisch (1893), however, was able to obtain positive chemotropic responses of Narcissus tazetta pollen tubes to their own and to foreign stigmas as well as other floral and ovule parts. Molisch postulated that tube growth is directed all the way to the egg by specific substances produced by the female. Miyoshi (1894) reported the presence of secretory drops at the micropylar exostome, and believed that they contained a sugar such as sucrose which was acting as the attractive agent. Miyoshi, therefore, believed that tube growth is mechanical in the style and chemotropic in the ovary.

An interesting feature of Molisch's and Miyoshi's ideas, as pointed out by Nawaschin (1898a), is that they both assumed pollen tubes are unhindered by female tissues in their growth, "...der Pollenschlauch
Lloyd (1899) agreed with Molisch (1893) that chemotropism is the important factor in directing tube growth, and with Nawaschin (1898a) that pollen tubes do not require unoccupied space in order to respond to such a stimulus as presupposed by Molisch and Miyoshi. Lloyd felt in addition that the distribution of the stimulant within transmitting tissue is a differential one, and that the synergids are both the source and the center of distribution for it.

Lidforss (1899) also supported the concept of chemotropic growth. Having demonstrated a positive response of Fritillaria tubes to dialyzed albumen, he concluded that the attractive agent is proteinaceous vice carbohydrate in nature. Kirkwood (1906), like Miyoshi (1894), believed the agent to be a sugar and, like Lloyd (1899), believed that the substance is produced by the embryo sac. Tokugawa (1914) divided pollen tube growth responses into two groups: saccharochemotropische and proteochemotropische.

Brink (1924) investigated nine species and demonstrated positive chemotropism in Antirrhinum and Narcissus, but not in Hippeastrum, Scilla, Nicotiana, Cucumis, Primula, Lythrum, or Vinca. He believed that tube growth is mechanical in the style, and that chemotropic growth, although unproven, is a possibility in the ovary. Tsao (1949) observed chemotropic responses in nine of thirty-six species. She found that pollen grains placed further than 1.0-1.5 mm from the gynoecial tissue being tested were not attracted, and she concluded that chemotropism is in part a function of floral age, the tissue being tested, and the time of year. Iwanami (1959) felt that lily pollen tube growth through the stigma is chemotropic, but that in the stylar canal it is partially mechanical.
and partially by gravity. He suggested also that a growth hormone type substance is involved in changing the direction of a tube's growth. Welk et al. (1965), however, believed that a major function of the secretory product in Lilium is chemotropic guidance of pollen tubes.

**Possible agents and their distribution**

The factors which promote or inhibit pollen germination, pollen tube growth, and chemotropic responses vary considerably among different taxa in regard to their incidence, chemical and physical properties, and their distribution in the gynoecium (Miki, 1961; Miki-Hiroshig, 1961). Among substances tested for chemotropic activity are various sugars, amino acids, dicarboxylic acids, growth hormones, and some minerals (Mascarenhas and Machlis, 1962b). After having determined that the factor is nonspecific, heat stable, resistant to dilute acid hydrolysis, small enough to be dialyzable, soluble in water and alcohol but not in acetone or ether, and that it has a growth-stimulating effect, Mascarenhas and Machlis (1962a, b, c, 1964) suggested that calcium is the general agent in higher plants. Mascarenhas (1966) later speculated that some other factor might be involved in addition to calcium.

Several studies have demonstrated a general axial concentration gradient of calcium in gynoecial tissues (Mascarenhas, 1966; Glenk et al., 1970; Day et al., 1971), and all have shown that the concentration increases basipetally. In Oenothera (Glenk et al., 1970), the highest concentration is in the placenta while in Gladiolus (Day et al., 1971), ovules contain the most calcium. Glenk et al. (1970) did not find any chemotropic response to calcium either in vitro or in vivo by Oenothera pollen tubes, but they did find that calcium ions are effective in
promoting pollen germination and tube growth. Calcium distribution in *Antirrhinum majus*, however, is not in an even, consistent gradient and this fact led Mascarenhas (1966) to the aforementioned conclusion that perhaps some factor in addition to calcium is involved in directing tube growth.

**Electrical fields**

Because calcium ions bond tightly to various cytoplasmic constituents, transcellular currents of this ion should establish relatively large cytoplasmic gradients and thus electrical fields (Jaffe et al., 1974). Pollen grains germinated in an electric field show a tendency for the tube to emerge from the aperture on the side of the grain which faces the cathode and for the tube to grow toward the cathode (Marsh and Beams, 1945). Weisenseel et al. (1975) studied the electrical fields around pollen tubes and found a current entering the growing tip and almost all of the tube behind it.

Each wetted pollen grain drives a steady current of a few hundred pico amps through itself. The current enters an ungerminated grain's prospective growth site and exits via the other end. After a grain germinates and forms a tube, the current enters most of the growing tube and leaves from the grain. Current continues to flow as long as the tube grows and growth is never found without current (Weisenseel et al., 1975). L. F. Jaffe et al. (1975), by way of ion substitution experiments, determined that calcium is one component of this growth-associated current. Potassium has been shown to be involved also (Weisenseel et al., 1975; Jaffe and Nuccitelli, 1977).
Calcium has been shown to accumulate in pollen grains as well as in the tip of growing tubes. With a zone of high accumulation extending 20-30 µm behind the tip, accumulation in the tip is 2-4 fold greater than in the bulk of the tube (L. A. Jaffe et al., 1975), but potassium ions enter the whole tube uniformly (Weisenseel et al., 1975).

Investigating other minerals, Yamada and Cho (1968) demonstrated with *Lilium longiflorum* that, while cobalt, copper, and zinc levels remain almost constant in the ovary, the stigma and style preferentially accumulate cobalt. The degree of acquisition increases with the age of the flower bud, and the concentration finally reaches a level of approximately eightfold greater than that found in pollen grains. Copper and zinc concentrations are about the same for both pollen and gynoecial tissues. In pollinated flowers, cobalt levels fall sharply about fifty hrs. After pollination while there is no change in unpollinated controls, Yamada and Cho concluded, by supporting the suggestion of Rosen (1961), that cobalt may improve the growth response of pollen tubes, but neither Rosen nor Yamada and Cho were able to correlate cobalt with directional growth.

Mascarenhas (1975) has summarized the state of affairs surrounding the isolation of the attractive agent: "...there is at best no unanimity of opinion concerning the chemical identity of the chemotropic substance(s) in the species studied."

**Branching and curvature**

In vivo branching of growing pollen tubes has been noted by Hofmeister (1859) for *Pothos* and *Hippeastrum*, by Benson (1894) in several Amentiferae,
by Nawaschin (1895, 1899) in *Juglans* and *Ulmus*, by Lloyd (1899) for several Rubiaceae, by Billings (1903) in *Carya*, and by Gore (1932) for cotton. Rosen (1961) has shown branching to occur in vitro with lily pollen growing on a partially purified stylar extract adjusted for osmolarity with sucrose. He correlated branching with chemotropism and postulated a possible mechanism by which pollen tubes respond to chemotropic stimuli. According to Rosen, "...the factor attracts pollen tubes up a concentration gradient by maintaining the plasticity of newly-formed wall material at the tip of the tube, so that in response to internal turgor pressure the tube bulges and grows in the direction of increasing concentration of the factor."

In light of the fact that curvature of tip-growing systems is brought about by bulging rather than bowing (Weisenseel et al., 1975) and that calcium-generated electrical fields are hypothesized to move vesicles of new membrane and new wall material to the growing tip (L. A. Jaffe et al., 1975), Weisenseel et al. (1975) state "...that local application of high Ca favors initiation or local expansion of these species pollen tubes. However, any direct action of high calcium on their walls would be expected to increase their rigidity *(by cross-linking acid polysaccharides) and thus inhibit local expansion. Hence, local application of high Ca may well act to favor local expansion of these cells by crossing the plasma membrane to refocus entry of endogeneous current." Weisenseel et al. footnote at the previous asterisk that personal communication with R. Cleland (U. Wash., Seattle) indicates that the latter's experiments on oat coleoptiles and pea stems show no direct effects of increased calcium on wall stiffness one way or the other. For further information
on pollen tube wall synthesis, see Engels (1973, 1974a,b,c) and Engels and Kreger (1974).

**In vitro versus in vivo growth**

The work of both Rosen (1961) and Weisenseel et al. (1975), however, was carried out in vitro, and differences in pollen tube growth have been shown between in vitro and in vivo conditions (Rosen, 1962). The ultrastructure of cotton pollen tubes growing in vivo has been described by Jensen and Fisher (1970). Differences in anatomy have been shown also for compatible vice incompatible situations (Pluijm and Linskens, 1966; Rosen and Gawlik, 1966). Tubes growing in incompatible gynoecia develop an anatomy similar to that seen in tubes growing in vitro. It has been postulated that compatible tubes change from an autotrophic mode of nutrition, with nutrients supplied by the parent grain, to a heterotrophic mode, with nutrients supplied by the stylar exudate. Pollen tubes growing in compatible styles are able to make the transition and maintain growth. Tubes growing in incompatible styles cannot change over and growth ceases when endogeneous nutrients are expired (Thimann, 1972; Vasil, 1974).

**Callose plugs**

As just noted, pollen tubes are nourished by first the pollen grain and subsequently by gynoecial exudates. Nutrient supplies from the pollen grain are effectively cut off from the growing tube by the formation of callose plugs in the tube (Benson, 1894; Miller, 1919; Brink, 1924). Such plugs were first described by Strausburger (1878) who named them Propfen. Soon after, Elfring (1879) also noted their occurrence as did Treub (1891) a bit later. But, whereas all three of these researchers thought the
plugs to be cellulosic, the correct chemical composition of plugs as callose was first determined by Mangin (1890).

Benson (1894) found that the first (proximal-most) plug may form as close to the grain as the tube-grain junction, or as far away as just behind the growing tip. Lloyd (1899) noted that in some instances the tube is empty on one side of the plug (pollen grain side) and that in other cases cytoplasm occurs on both sides. Miller (1919) concluded, "...formation of callose plugs in series serves to maintain the integrity of the vegetative pollen cell. It should be noted also that by cutting off the older part of the tube the plug limits the region from which the vegetative cell absorbs nutrient materials to the less exhausted portions of the style."

Brink (1924) demonstrated that pollen tubes severed between the grain and any plug are able to continue growth but that ones cut between the growing tip and the distal-most (youngest) plug cease growing immediately. Brink supported Benson's (1894) contention that plugs serve to isolate the growing tip of pollen tubes that undergo an overwintering or dormant period. In such cases, the plugs form as growth ceases and the tube tips are thus effectively isolated as sporelike entities. He also supported Miller's (1919) hypothesis that plugs allow the growing portion to draw its nutrients from only the richest source. Pollen tubes are generally ephemeral and deteriorate soon after fertilization, but in some cases they persist, e.g., *Probascidea* (H. L. Mogensen, Northern Arizona State University, personal communication, 1975), *Ulmus* (Shattuck, 1905), and *Stellaria* (Gibbs, 1907) among a few others (Maheshwari, 1950).
Tupý (1961) and Kumar and Hecht (1970) showed that there is a relationship between the growth rate of pollen tubes, callose plug formation, and the sugars present in the style. According to Yamada (1965), sugar reserves in the style are probably the main respiratory substrate in the earliest stages of pollen tube growth, and only later do proteins, polysaccharides, and lipids become important substrates. Tupý (1961) demonstrated that the glucose/fructose ratio changes in favor of glucose following fertilization.

Pollen tubes consume mainly sucrose, and, of this, primarily fructofuranose. The longer the tubes become, the faster they grow and the greater the amount of free glucose. Some glucose remains unused, accumulates in the pollen tubes, and then upon reaching a critical concentration, it condenses to callose (Tupý, 1961). Kumar and Hecht (1970) also showed that an increase in pollen tube development reduces callose plug formation as well as the content of endogeneous stylar sugar. They concluded that the amount of callose deposition depends on the pollen tube's ability to utilize the glucopyranose moiety of sucrose.

Postpollination Changes in Flowers

During the period that pollen tubes are actively growing, i.e., the interim between pollination and fertilization, the metabolic state of all parts of the flower changes. The style becomes a sink for organic materials from the stamens, the corolla and green calyx, and the ovary shows changes in the level of free amino acids, total RNA, and total protein. After a certain point is reached in the pollination-fertilization process, the ovary supersedes the style as the main sink (Linskens,
Sensitive stigmas

These postpollination responses of the flower, among some others, seem to be of fairly wide, and possibly universal, occurrence as will be shown momentarily. Among five families, however, there is a unique response, due in part to their stigma and stylar anatomy and in part to their physiology. Some 25-30 genera of the Bignoniaceae, Lentibulariaceae, Lobeliaceae, Martyniaceae, and Scrophulariaceae have what are known as sensitive stigmas (Newcombe, 1922, 1924). In these plants the stigmas are bilobed and are sensitive to mechanical and chemical stimuli (Sinyukhin and Britikov, 1967).

In mechanically stimulated but nonpollinated stigmas, the lobes start to move in 0.1 sec, are closed in 6-10 sec, and are reopened and ready to respond again in 17-22 min. The action potential from pure mechanical stimulation reaches only as far as the junction of the stigma lobes where the tissue giving rise to the motor response is located. In pollinated stigmas, however, the lobes begin to close in 3-7 min and, once closed, remain closed. The action potential spreads down the entire length of the style at 2.9 cm/sec, and by 60-90 sec after it reaches the ovary, the respiration rate of the ovary increases. Respiration returns to normal in 4-16 min and then slowly increases again to reach a maximum in 10-36 min (Sinyukhin and Britikov, 1967).

The ability of stylar tissues to block one action potential--mechanical stimulation--and conduct another--chemical stimulation--protects the ovary from the necessity of responding to incidental
mechanical irritation of stigmas, including irritation by insects bringing no pollen (Sinyukhin and Britikov, 1967). *Spathodea campanulata* seems to be unique among the unique in that it requires both pollen and mechanical pressure to cause complete closure of the stigma (Newcombe, 1924).

Sinyukhin and Britikov (1967) have shown that plants without sensitive stigmas, e.g., *Incarvillea*, *Zea*, and *Lilium*, exhibit an electrochemical action potential in response to pollination as well as those with sensitive stigmas. The action potential appears in the stigma first as a result of chemical interactions between the stigma and pollen. The potential then moves down the style, signals the ovary that pollen is on the stigma, and, as in species with sensitive stigmas, the ovary responds by changing its metabolism expressed as an increase in respiration.

**Hormone production**

Muir (1942) was one of the earliest to hypothesize that pollen tubes secrete an enzyme which activates growth hormones in the style and ovary. Since Muir, several investigators have shown postpollination increases in gynoecial levels of IAA, e.g., Lund (1956), Hall and Forsyth (1967), Forsyth and Hall (1969), and Thimann (1972), among others. In addition to IAA, IAAlike substances and GA are released by pollen also (Thimann, 1972; see Rosen, 1968 for a discussion of enzymes released by pollen grains on stigmas). In tobacco, IAA content in the apical half of the style increases rapidly during the first few hours following pollination and then drops after 25 hrs. Content in the basal half increases more
slowly, but continues to increase until 48 hrs. The IAA concentration increase in the ovary begins at about 50 hrs which correlates with pollen tube growth (Lund, 1956). The increase in IAA production has been implicated as causing a subsequent increase in gynoecial ethylene production (Hall and Forsyth, 1967; Forsyth and Hall, 1969; Lipe and Morgan, 1973).

As is becoming evident, one of the functions of the style is to act as a sensory organ. In this regard, changes in gynoecial hormonal metabolism are mediated by the style and information is transmitted to other floral parts (Gilissen, 1976). Petunia ovaries are stimulated to synthesize increased amounts of protein before pollen tubes reach the end of the style (Deurenberg, 1976). Ethylene production in Dianthus causes an increase in the ratio of sucrose to reducing sugars in the petals and an influx into the ovary of sugars and inorganic material. At least some of the influxing material is mobilized from the corolla (Nichols and Ho, 1975; Nichols, 1976). The postpollination movement of materials from floral parts into the ovary has been shown to occur also in tobacco (Tupý, 1961), orchids (Arditti, 1969; Arditti and Flick, 1976), and Petunia (Linskens, 1973, 1974a,c), among others. For greater detail on this topic, see Linskens (1973, 1974a,c), Arditti and Flick (1976), and Nichols (1976).

**Wilting**

Gilissen (1977) feels that the style also controls postpollination wilting of the flower. He showed that proteins, amino acids, and carbohydrates eluted from either foreign or dead self pollen did not cause any metabolic changes. Gilissen concluded that metabolic changes
resulting in wilting begin at the time pollen tubes penetrate the stigmatic surface and may possibly be induced by either a mechanical or a biochemical stimulus. He states further that corolla wilting can be considered as a means by which the flower communicates to the environment that the corolla is no longer functioning as a result of pollination.

History of Sexuality in Plants

Early history

According to Maheshwari (1950), Aristotle's student, Theophrastus, recounted from the writings of Herodotus (Fifth Century B.C.) the Arabian and Assyrian ceremonies of pollinating date palms. These early Greek tabloids are probably the earliest written record of observations on sexuality in plants.

It was not until almost some 2,000 years after Theophrastus that animal sperm was discovered by Leeuwenhoek (1677). Shortly following Leeuwenhoek's discovery, according to Schacht (1850), Malpighi (1681) discovered the ovule and embryo sac. Malpighi also examined pollen grains but regarded pollen as a useless secretion. Grew (1682) was the first to recognize stamens as being the male organs. He thought that just by falling on the stigma pollen transmitted a "vivific life-giving emanation," to the ovary and thus prepared it to produce fruit.

Through experiments with Mercurialis, Ricinus, and Zea, Camerarius (1694) produced scientific evidence that there must be an interaction between stamens and carpels to produce seed-bearing fruits. Camerarius' work was confirmed by Kölreuter (1761) who, in addition, gave a detailed
account of the importance of insects in floral pollination. Köhreuter also produced hybrids of *Dianthus*, *Matthiola*, *Nicotiana*, and *Hyoscyamus* and showed that, if a flower is pollinated simultaneously with pollen of its own kind and that of another species, the foreign pollen generally is not effective. This, he said, was the reason why hybrids are so rare in nature although they could be produced artificially.

Amici (1824) made the next significant advance by discovering the pollen tube. While working with *Portulaca oleracea*, he noted the pollen tube on the stigma and was able to trace it to the ovule, and Brongniart (1827) traced the pollen tubes in several different plants. In *Pepo macrocarpus* he saw the broken-off end of a pollen tube hanging from the micropyle. In the opinion of Schacht (1850), Brongniart misinterpreted the phenomenon for he regarded the pollen tube as a fertilizing tube through which the fertilizing contents were brought to the embryo sac. Once it arrived at the embryo sac, the tube's contents were taken up by the embryonal vesicle, a cell arising in the embryo sac. Amici (1830) wrote a letter to Mirbel in which he put forth the questions as to whether or not pollen tubes elongate bit by bit to finally reach the ovules, and whether or not there is one tube for each ovule. He answered his own questions in the affirmative.

Schleiden (1837) is reported by Coulter and Chamberlain (1909) to have traced pollen tubes in the gynoecia of members of many widely separated families. He claimed to have seen tubes enter the micropyle, pass into the embryo sac, and then they become the embryonal vesicle, the beginning of the embryo. Atkinson (1901) has indicated that the concept of the embryonal vesicle and the idea that angiosperm embryo sacs act as
incubators for young plants originated in the very early 1700's and were popularized by Schleiden and Schacht. Schleiden (1837) further believed that the contents of the tube not only gave rise to the embryonal vesicle, but that the end of the tube itself became the future plant, nourished at first by the embryo sac.

According to Coulter and Chamberlain (1909), Hartig (1842) described an egg in the embryo sac and claimed that the pollen tube carries a substance which fertilizes the egg, a view which Schleiden immediately opposed. In the same year, Amici reiterated his previous views and stated that in Orchis and other plants a preexisting cell in the embryo sac becomes the embryo via the influence of the pollen tube. Schacht (1850), who misinterpreted the pollen tube for the egg, sided with Schleiden and disagreed with Amici. Coulter and Chamberlain (1909) go on to say that von Mohl described the egg apparatus of Orchis and confirmed Amici's observations, and supported his conclusions. Both Schleiden and Schacht later admitted that their views were in error.

It remained for Hofmeister (1849), however, to show conclusively that embryos arise from a preexisting cell of the embryo sac and not the pollen tube. He was the first to describe the organization of the embryo sac as having basically two groups of cells. He considered the cells at the chalazal end to be prothallial and those at the micropylar end all to be capable of giving rise to a new sporophyte after fertilization. Hofmeister thus failed to distinguish between synergids and egg, but considering that he was working only with cleared specimens and free-hand sections, his observations are indeed remarkable.
In the years 1877-1881, great advancement was made in understanding embryo sac development through the works of Fischer, Guignard, Jonsson, Mellink, Strausburger, Treub, Vesque, Ward, and Warming. For reviews of these and other very early works, see Schacht (1850), Hanstein (1870), Warming (1877), Land (1900), Coulter and Chamberlain (1909), and Maheshwari (1950).

It was Strausburger (1879) who first demonstrated that the megaspore mother cell differentiates from a nucellar cell and then goes on to develop into the mature megagametophyte. Strausburger (1884) was also the first to describe syngamy. Nawaschin (1898a) demonstrated that both male gametes have a functional role in fertilization. Working with Lilium martagon and Fritillaria tenella, he was able to show that one sperm nucleus fuses with the egg (syngamy) to form the zygote, and the other fuses with the two polar nuclei (triple fusion) to form the 3n primary endosperm. Guignard (1901) described the movement of the male nucleus, on its way to affect triple fusion through the cytoplasmic strand connecting the polar nuclei and egg apparatus in Nigella, Damascena, Ranunculus, Cymbalaria, and Anemone. For a more in-depth coverage of the early history of sexuality in plants and for information on the history of early works on embryo and endosperm formation, see Guignard (1882), Sargant (1900), Coulter and Chamberlain (1909), Johansen (1945), and Maheshwari (1950).

Contemporary history

The first electron microscope studies of embryo sacs appeared in the early 1960's with Jensen's (1963) paper on cellular development in young
cotton embryos. Working with Dendrobium, Isreal and Sagawa (1964) demonstrated that the premeiotic archesporial cell has plasmodesmata. The plasmodesmata disappear at prophase leaving the megaspore mother cell isolated. In the same year, Pluijm (1964) studied the ultrastructure of the filiform apparatus in Torenia and concluded that this structure is of prime importance in the process of fertilization. His paper gave impetus to the yet unresolved controversy over the true function of the synergids.

Since these papers were published, there has been a gradual shift toward emphasizing the ultrastructural aspects of the reproductive process. This shift is due in part to the interest stimulated by these papers and in part to the advancements in electron microscopy since the early 1960's. In addition to the papers already mentioned, some of the other more important ultrastructural works dealing with embryology, to be discussed later, include Horner and Arnott (1965), Jensen (1965a), Jensen and Fisher (1967b), Linskens (1968), Cass (1972), and Mogensen (1972, 1973, 1975b). Recent reviews include Heslop-Harrison (1972) and Kapil and Bhatnagar (1975).

Although some observations on living embryo sacs have been made in the past, the use of living materials which allow direct observation while performing experimental techniques offers many exciting possibilities for future research. Careful use of vital stains and/or tracers coupled with time-lapse photomicrography or cinemamicrography could provide the innovative researcher with much useful information about megasporogenesis, megagametogenesis, pollen tube growth, and fertilization.
According to Erdelská (1974), there are three groups of plants which are good for studying living embryo sacs. The first of these is characterized by having relatively transparent ovules, e.g., *Monotropa hypopitys*, *M. uniflora*, *Cypripedium insigne*, *Calanthe veitchii*, *Dendrobium nobile*, *Brodiaea uniflora*, and *Jasione montana*. The second group has embryo sacs which can be isolated, either partially or completely, from the ovule. *Galanthus nivalis* is an example of this group. The third group, with embryo sacs that protrude beyond the micropyle, is exemplified by *Torenia fournieri*. See Erdelská (1968, 1974) for more details and other references on this subject.

**Theories on angiosperm megagametophyte evolution**

In regard to embryo sac evolution, there are numerous theories, and the subject has been reviewed well by Atkinson (1901), Maheshwari (1948), Battaglia (1951), and most recently by Cocucci (1973) and Sattler (1974). The earliest of the three most prominent theories is that of Porsch (1907).

Porsch's Theory states that the angiosperm embryo sac has been derived through the reduction of the gymnosperm female gametophyte to two archegonia with a concurrent loss of all prothallial tissue. The two polar quartets of nuclei represent the two archegonia as follows: egg homologous with egg; two synergidals or two antipodals homologous with two neck cells; each polar nucleus homologous with a ventral canal cell. This theory has been criticized mainly on the grounds that it does not satisfactorily account for the homologies in the mitotic divisions and in their later derivatives, e.g., embryos originating from synergidals, which, according to Porsch's Theory, would be equivalent to embryos
developing from neck cells, an unknown phenomenon.

The second theory is that of Schürhoff (1919, 1926, 1928). Schurhoff claimed that the egg apparatus is analogous to two archegonia and the other cells are prothallial. The egg and one synergid constitute one archegonium with the synergid equivalent to the ventral canal cell. The other synergid and the micropylar polar nucleus constitute the second archegonium. This theory has been refuted on the basis that the egg and synergids are not sister nuclei: the two synergids are sisters as are the egg and micropylar polar nucleus.

The third theory, the Gnetalian or Archegonial Disappearance Theory is not attributed to any one person, but rather has been derived from the opinions of Hofmeister, Strausburger, and others (Maheshwari, 1948) and has been supported by various widely accepted authors, e.g., Maheshwari (1948) and Battaglia (1951). According to Battaglia (1951), the Gnetalian Theory does not assume a direct derivation of the angiosperm embryo sac from the Gnetales, but that the reduction of the female gametophyte in both the Gnetales and angiosperms followed a more-or-less parallel course leading to a complete loss of archegonia. Proponents of this theory claim that there is no archegonial homology between gymnosperms and angiosperms and that the reduction of the female gametophyte from gymnosperms to angiosperms is characterized by the disappearance of the archegonium.

According to Maheshwari (1941), the monosporic 8-nucleate type of embryo sac is the most primitive and is the one from which all others can be derived with the least difficulty. For more detail on embryo sac phylogeny and classification systems, see Coulter (1908), Palm (1915),
Rutgers (1923), Shadowsky (1925), Chiarugi (1927), Modilewski (1929)
Schnarf (1936), Maheshwari (1937, 1941, 1950, 1955), Davis (1966),
Cocucci (1973), Sattler (1974), Philipson (1974), and Favre-Duchartre
(1976).

Although embryological characters generally are constant at the
familial level, a few examples of intrageneric, and even intraspecific,
variation do exist, viz. in patterns of megagametogenesis (Palser, 1975).
As an example of the extreme condition, several species of orchids exhibit
variation within an individual (Abe, 1972). To appreciate the nature of
this variation, Herr (1967) states, "...achievement of a full perception
of variation in the Polygonum type of megagametophyte will require atten­
tion to the following developmental features: a) ovule initiation;
b) archesporium; c) megasporocyte; d) meiosis I; e) dyad; f) meiosis II;
g) tetrad; h) functional megaspore; i) two, four and eight nucleate
megagametophytes; j) mature megagametophyte."

Use of the term embryo sac

In keeping with the proposal of Johansen (1941) to abandon use of
the term, embryo sac mother cell, the term will not be used in this paper.
Johansen (1941) also proposed that the term, embryo sac, be restricted
in its use to refer to the remnant of the former megagametophyte. In
other words, the term should refer to only the structure which contains
the embryo and, if present, the endosperm. In view of the fact that an
embryo is not present until after fertilization, Johansen's proposal is
sensible and will be adhered to for the remainder of this paper. For
stages prior to fertilization then, the term, megagametophyte, will be
used exclusively.

Megasporogenesis

According to Heslop-Harrison (1972), an ovule may be defined as "...an integumented megasporangium, formed on the surface, or near the margin, of a carpel." Swartz (1971) defines it as "The unfertilized young seed in the ovary; the structure which, after fertilization, develops into a seed; the megasporangium of a seed plant which later develops into a seed; a rudimentary seed." As will become evident later, these definitions, particularly the former, are rather oversimplified and not completely accurate, but taken together they do provide some idea as to the nature of an ovule.

Archespore

The angiosperm megasporangium is hypodermal in origin, being derived from the outermost layer of the periblem. It is thus an embedded organ which becomes superficially distinct through the growth of cells beneath and around it (Coulter and Chamberlain, 1909). The archesporial tissue is also hypodermal in origin. Usually one cell, but not uncommonly two or even several cells, located immediately adjacent to the epidermis becomes distinct due to its increase in size and cytoplasmic density. This cell(s), the archesporial cell(s), also develops a more distinct nucleus (Maheshwari, 1950), and often assumes a tetrahedral configuration. The archesporial cell is usually the terminal one of an axial row of cells of the nucellus, especially in monocotyledons which commonly have small ovules with large cells. In large ovules with small cells, particularly in dicotyledons, such axial rows are not evident and proof of an apparent
terminal position is not readily demonstrable (Mottier, 1895).

Parietal cells

The archesporial cell may function directly as the megaspore mother cell (MMC) or it may divide to form a primary parietal cell and a primary sporogeneous cell (Maheshwari, 1950). The general evolutionary trend has been toward suppressing this division so that higher groups tend to lack a parietal cell (Coulter and Chamberlain, 1909).

Schnarf (1929) recognizes six different types of development between the archesporial cell stage and the megaspore mother cell stage. Schnarf's classification system is based on the activity, or lack thereof, in the formation of parietal cells. Neither Schnarf's system nor any of the several other such schemes have become popular. Ovules are referred to simply as having a parietal cell(s) or not having a parietal cell(s).


Behavior of the primary parietal cell is highly varied: it may remain undivided, or it may divide anticlinally and/or periclinally to form a variable number of wall layers around the archesporial cell (Coulter and Chamberlain, 1909; Maheshwari, 1950). While the more common condition among those plants which do have parietal cells is the formation
of either one or two parietal cells by paraclinal division(s), *Ornithogalum gussonei* forms one or two rows of parietal cells (Zabińska, 1972). In *Euphorbia geniculata*, the primary parietal cell divides several times in both the paraclinal and anticlinal planes to form a nucellar beak (Bor and Kapil, 1975). See Coulter and Chamberlain (1909) and Schnarf (1929) for more detailed accounts of parietal cell activities. The topic of nucellar beaks is discussed in the section on nucelli.

As will become evident in later sections, differentiation of the archespore and/or the onset of meiosis is often used as a reference point in ovule development. Of particular note in this regard are integument initiation and curvature of the ovule. As an example of the latter situation, the ovule of *Forstera tenella* is said to be almost completely anatropous by the time the archesporial cell is distinguishable (Philipson and Philipson, 1973). In most instances, however, ovule curvature does not begin until later and is commonly coincident with archespore differentiation (Coulter and Chamberlain, 1909; Maheshwari, 1950). It should be realized, however, that in not all taxa do all ovules within a given ovary develop synchronously, e.g., *Ornithopus* (Wojciechowska, 1975).

**Megaspore mother cells/meiosis**

The archesporial cell matures into the megasporocyte which then undergoes meiosis. In *Ammannia* the MMC nucleus is characteristically located toward the micropylar end of the cell, and meiosis occurs there (Smith and Herr, 1971). As a general rule in angiosperms, the first meiotic division is always transverse and the second usually is also. The second division, however, may result in a T-shaped tetrad. The two
cells lying side-by-side may be at either pole, but are more commonly in the micropylar end. The second division may also produce an oblique, vice a perpendicular, wall in the micropylar end (Maheshwari, 1950). The most common condition is the formation of a linear tetrad (Maheshwari, 1941). Occasionally the micropylar cell of the dyad fails to divide resulting in only three megaspores after the chalazal cell divides, e.g., Euphorbia geniculata (Bor and Kapil, 1975) and many others (Schnarf, 1929; Maheshwari, 1950). See Lloyd (1899) for a review of 19th Century papers on meiosis and cell division, and see Bennett (1973), Bennett et al. (1973a,b), and Bennett and Stern (1975a,b) for a more recent discussion of female meiosis.

In a somewhat questionable report, Billings (1937) claimed that the archespore of Isomeris arborea develops directly into the megagametophyte without first producing megaspores, except for one case in which he did observe megaspores. He thus proposed the absence of meiosis as being the normal condition for Isomeris. He further stated that there is a complete lack of polar nuclei and antipodals, that there is no true egg present, and that the pollen tube neither enters the megagametophyte nor does it discharge its contents. Billings suggested that the pollen tube causes a mechanical stimulation and claimed that his findings were the first recorded, well-documented account of a well-established, wild species exhibiting monoploidy.

**Cytoplasmic inclusions** Among some of the early accounts of megasporogenesis there are several reports on the occurrence of rather peculiar cytoplasmic features in the MMC, particularly in those plants
with *Fritillaria* type development. Flint and Johansen (1958) divided these features into three groups: 1) cytoplasmic radiations; 2) heavily staining cytoplasmic globules, and 3) thick perinuclear layers of cytoplasm. Members of the first category include: *Tulipa gesneriana* (Bambacioni and Giombini, 1930), *Gagea graminifolia*, *G. ova*, and *G. ternera* (Romanov, 1936), *Gagea fascicularis* (Joshi, 1940), and *Lilium neilgherrense* (Subramanyam and Govindo, 1949). *Lilium martigon* (Sargant, 1896) exemplifies the second group, while *Lilium henryi* (Cooper, 1935), *L. michiganense* (Conant and Haquist, 1944), and *Erythronium* (5 spp.) (Smith, 1955) are members of the third class.

Flint and Johansen (1958) studied *Lilium pardalinum*, *L. columbianum*, *L. regale*, *L. michiganense*, *Fritillaria mutica*, and *Erythronium oregonum* and found that at leptotene of prophase I, there are extrusions from one area of the nucleolus. At the end of the extrusion process, there is very little stainable nucleoplasm when formerly there had been a considerable amount. A number of other early investigators have reported nucleolar extrusions, and this literature is reviewed by Gates (1942). Soon after the extrusion process begins, radiations appear in the cytoplasm. For the most part, these radiations extend from the nuclear membrane to the plasmalemma. Flint and Johansen (1958) concluded that the radiations either hold nuclei in place or direct their movements. For more on theories of nuclear movement mechanisms, see Romanov (1938, 1970), Fagerlind (1938), and Helmquist and Grazi (1965).

In addition to their first premise, Flint and Johansen (1958) believed that the heavily staining cytoplasmic globules and the thick perinuclear layers of cytoplasm comprising the other two categories of
cytoplasmic structures are actually radiations which have been distorted by chromic compounds in the fixing solutions. Radiations similar to those of Flint and Johansen have been identified ultrastructurally as being groups of parallel ER cisternae by Rodkiewicz and Mikulska (1965).

The MMCs of Sonneratia are reported to contain some sort of unique, unidentified bodies (Maheshwari, 1941). In Bupleurum, some MMCs have extensive paracrystalline cytoplasmic inclusions. These inclusions are oriented axially and, in B. dianthifolium, they remain in the chalazal megaspore while, in B. canescens, fragments are found in all four megaspores (Francini-Corti and Cecchi-Fiordi, 1973). A cytoplasmic "compound mass" occurs at the chalazal end of the MMC of Cytisus (Corti, 1950). Corti concluded that this mass resembles similar such masses found in some gymnosperms and that it allows the cell to undergo meiosis. He stated further that this mass may also be responsible for the chalazal megaspore becoming the functional one.

Ultrastructure and organelle activity In the MMC of Pisum, the pars morpha phase of the nucleolus occupies most of the center of the nucleolus. The fibrillar and granular components of the nucleolemma are evident together in early prophase, but they do separate at late pachytene. At mid-pachytene, the nucleolus moves from the center of the nucleus to the nuclear membrane. The pars morpha phase disappears at this stage and the nucleolus remains at the nuclear membrane until it disappears completely at metaphase. The nuclear pores disappear in the region where the nucleolus adheres to the nuclear membrane but remain abundant elsewhere. The intra-membrane space between halves of the nuclear envelope are wider next to the nucleolus than elsewhere on the
Dickinson and Heslop-Harrison (1977) report that during female meiosis in angiosperms a large part of the ribosome population is eliminated in an early period. Immediately before the ribosomes disappear, a goodly proportion of the meiocyte cytoplasm is segmented by double- or multiple-unit membrane profiles. Significantly, this segmented cytoplasm is unaffected by the agents responsible for the degradation of ribosomes. The authors claim also that these events are reflected in the organelle populations where cycles of dedifferentiation and redifferentiation occur. Microbodies are present both before and during ribosome degradation in females, but they are not so evident in males. New ribosomes form poly­somes and synthesize protein as paracrystalline inclusions. The new ribosomes are produced by supernumerary nucleoli at pachytene (Dickinson and Heslop-Harrison, 1977).

In Onoclea sensibilis, a fern, anaphase II is characterized by the formation of a compact plate of mitochondria across the MMC. Part of the plate is composed of amyloplasts, but they are not as localized in their concentration as are the mitochondria. Chromosomes are found outside of the plate area and lipid bodies occur randomly throughout the cell (Marengo, 1977). A similar condition also exists in Polypodium aureum (Marengo and Marengo, 1972), but formation of such a mitochondrial plate has never been reported in angiosperms.

**Callose**  
Although it has been known since the 1800's that callose has been associated with microsporogenesis, it was not until the early 1900's that callose was recognized as being involved with megasporogenesis as well. Graustein (1930) was the first to identify callose in association
with megaspores in Selaginella, and Horner and Beltz (1970) demonstrated that only the functional megaspore of Selaginella is surrounded by callose. To date, only one gymnosperm, the cycad Encephalartos poggei, has been shown to produce callose during megasporogenesis (De Sloover, 1961; Waterkeyn, 1961).

The report by Rodkiewicz (1967) of callose in the megaspore of Antirrhinum majus is the first such record in angiosperms. The following year, Rodkiewicz and Gorska-Brylass (1968) demonstrated that callose deposition in Dactylorhiza maculata begins at mid-prophase I. The MMC is completely invested by metaphase I, with the heaviest deposits occurring between meiosis I and II. The first report of callose in grasses is that of Schwab (1971) for Diarrhena, and it has since been reported to occur in Agrostis (Maze and Bohm, 1974).

In a survey of forty-three species from fourteen families, Rodkiewicz (1970) found that all of the thirty-nine species with a monosporic, and the one with a bisporic, pattern of megagametogenesis formed callose. The three species with a tetrasporic pattern did not. From this and previous data, Rodkiewicz (1968, 1970) concluded that monosporic species generally form callose whereas tetrasporic species do not. A second generalization concerning callose deposition is related to the Oenothera vice the Polygonum type of megagametophyte development. During early prophase, callose is deposited around almost the entire megasporecyte cell wall. A region is found at the micropylar apex in the Oenothera type and at the chalazal apex in the Polygonum type, however, in which only a minor amount of callose, if any at all, is laid down. As megasporogenesis progresses, callose is deposited in the dyad and tetrad
cross walls, but the apices originally with little or no callose remain essentially unchanged (Rodkiewicz, 1968, 1970; Kuran, 1972; Noher de Halac and Harte, 1975). In some monosporic types, especially among members of the Polygonum type, the entire MMC is surrounded with callose at meiosis I. This callose then undergoes localized dissolution at the chalazal end of the spore (Rodkiewicz, 1975).

In the orchid, Epipactis, there are numerous areas in the chalazal wall without callose which give the wall a sievelike appearance (Rodkiewicz, 1975). Epilobium palustre, another orchid, has patches in the meiotic cross walls that are devoid of callose. This allows for free movement of materials between the megaspores (Rodkiewicz, 1973). A similar situation also occurs in Fuchsia hybrida (Rodkiewicz and Kadej, 1974). For more information on the general occurrence of callose in plants, see Mangin (1890) and Currier (1957), and on callose as related to megasporogenesis, see Rodkiewicz (1968, 1970), Rodkiewicz and Kadej (1971), Rodkiewicz and Bednara (1974), and Noher de Halac and Harte (1975).

**Functional megaspore**

As a general rule, three of the four megaspores produced by meiosis degenerate. The remaining cell, the functional megaspore (FM), germinates and undergoes mitotic divisions to produce the female gametophyte. Sometimes in Ornithogalum gussonei, however, the two chalazal megaspores persist and only the micropylar two degenerate (Żabińska, 1972). Twin megagametophytes have been reported also in O. umbellatum (Desole, 1947; Czapik, 1966). In Galanthus nivalis, some ten percent of the ovaries contain one or more ovules with two FMs, but the second megagametophyte
does not always develop completely (Erdelská, 1975b). In addition to Ornithogalum and Galanthus, there are many other exceptions to the rule (Schnarf, 1929).

Another general rule, also with many exceptions of which most notable are those plants with the Oenothera type megagametophyte development, is that the chalazalmost megaspore becomes the FM (Schnarf, 1929; Maheshwari, 1937, 1950). For example, in Vicia faba, it is the second megaspore from the chalazal end which becomes the FM (Mitchell, 1975). To account for the success of the chalazal megaspore, Dixon (1936) proposed the diffusion of a mitosis-inducing hormone from the chalaza while Koernicke (1901) and Erdelská (1975b) attribute its success to its greater proximity to the hypostase, "...through which nutrients come to the embryo sac from the termination of the vascular bundle in the funiculus" (Erdelská, 1975b).

Lateral walls of the FM lose callose before those of the other three megaspores (Rodkiewicz and Bednara, 1974), and FMs are commonly characterized by the beginning of cell vacuolation (Maheshwari, 1937). For example, the FM of Ornithogalum gussonei has two polar vacuoles with its nucleus in the center of the cell (Żabińska, 1972). In Epilobium, starch is highly polarized at both poles of the FM while the dictyosomes are perinuclear (Rodkiewicz and Bednara, 1974).

According to Ikeda (1902), the FM of Tricyrtis hirta grows rapidly and the chalazal end penetrates into the nucellus and chalaza. Ikeda concluded that this portion of the FM serves as a haustorium for the developing megagametophyte. Megaspore haustoria have also been reported for Sedum sempervivoideae and Rosularia pallida (Mauritzon, 1933) and Galium lucidum (Fagerlind, 1937). The megaspore of Siparuna eggersii is
claimed by Heilborn (1931) to follow a pollen-tubelike development.

Megagametogenesis

According to the terminology of Maheshwari (1937), the first mitotic division of the FM results in the formation of a primary chalazal nucleus and a primary micropylar nucleus. The number of mitotic divisions required to produce the mature megagametophyte varies according to the type of developmental sequence a given plant follows. In the Polygonum (normal) type, there are three and in the Fritillaria type there are four (Maheshwari, 1950). Lily, long thought to be of the Polygonum type and thus used to exemplify typical megagametogenesis in angiosperms, was shown to be of the Fritillaria type by Bambacioni (1928). She confirmed this first report with subsequent work (Bambacioni and Giombini, 1930; Bambacioni-Mezzetti, 1931), and Cooper (1934) also verified it in Lilium henryi and several other species of lily. For ultrastructural information on megagametogenesis in Lilium, see Rodkiewicz and Mikulska (1963, 1965, 1966a,b, 1967) and Mikulska and Rodkiewicz (1964, 1965, 1967).

Among some orchids, a phenomenon known as strike occurs (Abe, 1972). Strike is the condition in which either the primary chalazal nucleus fails to divide or in which it does divide but only one daughter nucleus divides resulting in 1-3 (vice 4) chalazal nuclei. Six nucleate megagametophytes in orchids can also occur by fusion of two spindles appearing in the last division of the two chalazal nuclei in the four-nucleate megagametophyte. When mature, such megagametophytes have a typical egg apparatus, one-1n and one-2n polar nuclei, and one-2n antipodal (Abe, 1972).
Integuments

According to Balfour (1901), as an outgrowth of the sporangial primordium, the tegumentary system of an ovule is of variable origin and development. He regarded its primary function in angiosperms to be a water jacket and food store which evolved in response to special demands for water involved in the seed habit. More recently, Ortu (1970) has discussed their significance in terms of protection against radiation. Schleiden (1837) believed that the integuments originate in the chalaza while Bor and Kapil (1976) proposed that the outer integument is a derivative of the funiculus.

Boesewinkel and Bouman (1967) claim that in some Juglandaceae the integuments arise as two halves or valves which are originally free distally but later become fused proximally. Working with the same taxonomic group, Heel and Bouman (1972) concluded that "There is no escape from the fact that the integument in these plants grows like two fusing lobes." In Trifolium, Rembert (1977) stated that the inner integument is a lobed structure in its initial development, and he thus supports the contention that integuments originated from a fusion of parts. Heel (1970) also reported the occurrence of lobed integuments. A careful ontogenetic investigation of these plants would make an interesting study, especially in light of the fact that extant literature does not have a sequence of micrographs illustrating the development of free primordia and their later fusion.

The absence of an outer integument on the funicular side of bitegmic ovules was believed by Schleiden (1839) to be the result of congenital fusion between outer integument and funiculus. Schleiden's view has been
supported by such notables as Eames (1961) and Fahn (1974) among others. Bor and Kapil (1976) of A. D. J. Meeuse's school, however, oppose this view as noted previously and claim that "...inner and outer integuments are not homologous appendages from an ontogenetic point of view and the concept of 'congenital fusion' must be disregarded as an explanation."

Initiation The stage of ovule development during which integuments are initiated is variable. In *Pelargonium* (Tsai et al., 1973), integuments are initiated prior to archespore differentiation while in *Lycopersicon* (Cooper, 1931) integument initiation and archespore differentiation are concurrent events. In what is probably the most common condition, initiation occurs during the early stages of meiosis, e.g., *Ornithopus* (Wojciechowska, 1972a), *Trifolium alexandrinum* (Krupko, 1973), *Brassica oleracea* (Mackiewicz, 1973), and *Chlorogalum* and *Schoenolirion* (Cave, 1974). Generally the inner integument is initiated first and completes its growth first, but in *Decaisnea insignis* (Swamy, 1953) and *Ornithopus* (Wojciechowska, 1972a) the integuments are initiated simultaneously. The outer integument of *Ornithopus* grows faster than does the inner (Wojciechowska, 1972a). Integuments usually attain their full extension before megagametogenesis is complete, but, in *Caltha palustris* (Mottier, 1895), the gametophyte is often mature before the integument reaches the nucellar apex.

Number The number of integuments per ovule is variable as well as the time of their initiation. Of the 319 families surveyed by Davis (1966), 208 are bitegmic, 90 are unitegmic, and in 15 families the number of integuments is generic or specific. Most monocots and dialypetalous dicots have two integuments (Houk, 1938), and most sympetalous dicots
(Coulter and Chamberlain, 1909; Houk, 1938) as well as the Umbelliferae and Leguminosae have only one (Guignard, 1881; Coulter and Chamberlain, 1909). Some members of other dicotyledonous families may have only one integument also, e.g., Solanaceae (Cooper, 1931) and Ericaceae (Yamazaki, 1975).

The list for taxa with ategmic ovules varies according to the author. Davis (1966) says only that some species are ategmic, and Coulter and Chamberlain (1909) state that the two families, Loranthaceae and Balanophoraceae have no integuments. The list of Wettstein (1935) is the longest and includes the Santalaceae, Loranthaceae, Balanophoraceae, Olaceae, saprophytic Gentianaceae, and species of Crinum. Toilliez-Genoud (1965) also reported naked ovules in Crinum, as did Lloyd (1899) for Houstonia. The single integument of Alchemilla alpina (Murbeck, 1901) coalesces so completely with the nucellus that the ovule resembles a naked nucellus. Similar conditions are recorded for some Rubiaceous genera (Lloyd, 1899) including Coffea which Houk (1936) originally misinterpreted as being naked, but later (Houk, 1938) demonstrated the presence of an integument fused to the nucellus.

Anatomy and ultrastructure As a general rule, ovules do not have stomates, but stomates have been reported to occur in the outer integument of ovules from Nerine curvifolia (Schlimbach, 1924) and Gossypium hirsutum (Stewart, 1975). The outer integument of Hymenocallis occidentalis produces a well-developed chlorenchyma in addition to stomates (Flint and Moreland, 1943). A few additional examples are cited in Maheshwari (1950).

While in Gossypium the integumentary epidermal cells produce the cotton fibers of commerce (Jacob, 1943), those of Plantago ovata produce large quantities of mucilage subsequent to a large increase in dictyosome
vesicle number and size (Hyde, 1970). Epidermal cells of the outer integument of _Acer saccharinum_ form a network of loose, hairlike cells toward the micropylar end of the ovule (Haskell and Postlethwait, 1971), and, in _Nerium indicum_, it is the epidermal cells of the inner integument which produce hairs around the micropyle (Devi and Narayana, 1975). A caruncle is formed from the outer integument of _Acalypha rhomboidea_ (Landes, 1946).

The outer layer of the outer integument in _Polygonum pensylvanicum_ (Neubauer, 1971) contains lightly pigmented cells, and, except for the micropylar region, tannins are found in the outer layers of the outer integument in _Tripetaleia paniculata_ and _T. bracteata_ (Yamazaki, 1975), _Forstera tenella_ (Philipson and Philipson, 1973), and in the epidermis of both integuments of _Pelargonium_ (Tsai et al., 1973). Tubular para-crystalline structures occur in the cytoplasm of inner integument cells of _Diplotaxis eurcoides_ (Cresti et al., 1974). The structures seem to be derived from enlarged cisterns (sic) of RER, and Cresti et al. (1974) concluded that the structures are probably proteins which are stored for later use in embryo nutrition.

In _Nerium oleander_, walls of the integumentary cells in direct contact with the nucellus increase in thickness when the nucellus begins to degenerate. This and other changes progress on into the adjacent 2-3 cell layers of the integument. The portion of the wall in contact with the plasmalemma develops a peculiar layer characterized by the presence of numerous membranelike structures. These structures are flat saccules derived from the plasmalemma, and the cell wall zone containing them has been termed the paraplasmic layer (Gori, 1971, 1973). Similar structures
have been reported in the cell walls of an alga, *Chara vulgaris* (Barton, 1965).

In *Quercus*, the highest concentration of food reserves within the ovule is in the outer integument. The distribution of both carbohydrates and lipids is similar—very high in the outer integument and scarce in the inner. As the megagametophyte develops, there is a steady decrease in the size and number of starch grains from the micropylar to the chalazal end of the outer integument (Mogensen, 1973). The integuments of *Tricyrtis* also contain the greatest quantity of stored starch (Ikeda, 1902), but in *Agave* the integuments apparently have only a minor function in nutrient storage (Tilton, 1974).

Ultrastructure of the outer integument in *Quercus* is characterized by numerous plasmodesmata in the cell walls but plasmodesmata are in the inner integument (Mogensen, 1973). Plasmodesmata are more frequent in the chalazal end than they are in the micropylar end of the integuments of *Agave* (Tilton, 1974). In *Quercus*, epidermal walls are thin and outer integument walls are thick while those of the inner integment are of uniform width (Mogensen, 1973). Both integuments and the nucellus of *Agave* (Tilton, 1974) and *Triticum* (Morrison, 1975) are covered by a lamellate cuticle.

The integment of *Helianthus* is divided into two areas, the cell walls of the inner area being thick and those of the outer area thin. Walls of inner area cells adjacent to the integumentary tapetum begin to increase in thickness at an early stage in megagametogenesis. Wall deposition also occurs in integumentary tissue chalazad the megagametophyte which makes for a gradual transition into the inner core of thick-
walled, elongate cells. During the MMC stage, tissues of both the integumentary tapetum and the integument proper appear similar in size and content, but the integument proper cells are slightly more vacuolate. At megagametophyte maturity, tapetal cell walls adjacent to the megagametophyte are very thick except in those cells at the micropylar end of the tapetum where such thickening does not occur (Newcomb, 1973a).

In *Quercus*, the outer integument has abundant mitochondria and ribosomes (both free and bound types), and the ER is sometimes found to be continuous with the nuclear envelope. Dictyosomes are not common. In the inner integument, mitochondria and ribosomes are abundant also, but examples of ER-nuclear envelope continuity were not found. Plastids without starch are common and dictyosomes are few (Mogensen, 1973).

**Integumentary tapetum** Frequently, a definite nutritive jacket, consisting of one or more layers, invests the megagametophyte as alluded to in the previous discussion of *Helianthus*. For the most part, such nutritive jackets are a single layer of cells derived from the integument and are thus termed the integumentary tapetum. In *Armeria*, however, it is derived from the nucellus and in *Erodium* one layer is derived from the nucellus and one from the integument (Coulter and Chamberlain, 1909). Integumentary tapeta occur in 57 families (Davis, 1966) but are especially common in the Scrophularaceae, Stylidaceae, and many Sympetalae (Coulter and Chamberlain, 1909). Selected examples of genera having integumentary tapeta include *Solanum* (Young, 1923), *Hyoscyamus* (Svensson, 1926), *Androcymbium* (Cave, 1967), *Ammannia* (Smith and Herr, 1971), *Calendula* (Vassiljev and Plisko, 1971), *Ornithopus* (Wojciechowska, 1972a), *Forstera* (Philipson and Philipson, 1973), *Helianthus* (Newcomb, 1973a,b), *Jasione*
(Erdelská, 1975a), and Bellis (Engell and Petersen, 1977). In Jasiona, the integument is separated from the megagametophyte at an early stage by a cuticle, but the cuticle later disappears around the submicropylar portion of the megagametophyte (Berger and Erdelská, 1973; Erdelská, 1975a). The megagametophyte of Torenia is also cutinized (Pluijm, 1964).

Steffen (1955) reported the occurrence of 4, 5, 16, and 32 n nuclei in the integumentary tapetum of Pedicularis. Other such reports on DNA content of integumentary tapetal cells are lacking in extant literature.

In some crucifers, Goldfluss (1899) demonstrated that integumentary cells around the antipodals change as the megagametophyte matures. She termed this area the pseudochalaza and postulated that these altered cells are a source of nutrition for the megagametophyte and the embryo. Engell and Petersen (1977) reported a similar finding in Bellis perennis. Also in crucifers, Prasad (1975) found that integumentary tapetal cells, along with the megagametophyte itself, become greatly elongate if fertilization does not occur. Following fertilization in Plantago lanceolata, integumentary cells are invaded and destroyed by haustoria produced by the embryo sac (Vannereau and Mestre, 1975).

**Micropyle**

In the vast majority of angiosperms, a micropyle is present and is formed by either one or both integuments. The ovules of Gastrodia elata, an orchid, have no micropyle and the pollen tubes grow directly through the intercellular spaces of the micropylar nucellar cells (Abe, 1976). Sterling (1974b) reported that sometimes a micropylar opening is not
evident in *Camptorrhiza* and *Ornithoglossum*. The ategmic taxa mentioned in the preceding section are also an exception. The micropyle of *Coffea* is reported to be very narrow and easy to overlook (Houk, 1938). Davis (1966) surveyed 189 families and found that 74 use both integuments to form the micropyle, 38 use only the inner, and 4 use only the outer. In the remaining 73 families, this character is generic.

Examples of plants using only the inner integument are *Ornithogalum gussonei* (Zabińska, 1972), *Agrostis* (Maze and Bohm, 1974), and *Agave* (Tilton, 1974). Plants using only the outer integument are *Colletia* (Laguna and Cocucci, 1971), *Hydrobryopsis* (Arekal and Nagendran, 1975b), and *Hydrobryum* (Nagendran et al., 1976). *Euphorbia* (Lyon, 1898), *Lysichiton* (Campbell, 1899), *Sparganium* (Asplund, 1972), *Magnolia* (Boer and Bouman, 1972), *Ochna* (Chikkannaiah and Mahalingappa, 1974), and *Erythrina* (McNaughton, 1976) are among those plants which employ both integuments in forming the micropyle.

In some instances where the micropyle is formed bitemgically, the endostome, formed by the inner integument, and the exostome, formed by the outer integument, do not correspond. The result is a micropyle which is angled, curved, or zig-zag, e.g., *Curatella* and *Dillenia* (Dickinson, 1968), *Ammannia* (Smith and Herr, 1971), *Paspalum* (Chao, 1971, 1977), *Phytelephas* (Uhl and Moore, 1971), and *Ornithopus* (Wojciechowska, 1972a). It is common for one—e.g., *Phorium* (Cave, 1955), *Blandfordia* (Fulvio and Cave, 1964), and *Chlorogalum* and *Schoenolirion* (Cave, 1974)—or both integuments—e.g., *Pelargonium* (Tsai et al., 1973)—to have a greater number of cells around the micropyle than in other areas of the integuments.
Integumentary cells lining the micropyle of *Tricyrtis* are not cuticularized and at the time of fertilization their starch content is at a maximum (Ikeda, 1902). In that the starch grains disappear soon after fertilization, Ikeda concluded that they are related to pollen tube nutrition. Similarly, the inner integument cells in the micropylar region of many Alsinoideae show a considerable increase in size and staining intensity, and the contents are thought to be used up in nourishing growing pollen tubes (Gibbs, 1907).

The micropyle of *Senecio aureus* contains a mucilaginous substance that is believed to be secreted by the synergids (Mottier, 1893), and, in both *Diodia* and *Richardsonia*, cells lining the micropyle are characterized by somewhat thicker, more deeply staining walls than those of other integumentary cells (Lloyd, 1899). The micropyle of *Paspalum* is filled with a PAS positive substance which Chao (1971, 1977) believed is involved in directing pollen tube growth. Growth of the outer integument following fertilization occludes the micropyle in some members of the Cannabineae (Zinger, 1898), *Blandfordia* (Fulvio and Cave, 1964), and others (Maheshwari, 1950).

In addition to its role in fertilization (Němec, 1931; Tchuome, 1966), the micropyle apparently has other important physiological functions. According to Vlasova (1973), nuclear volume and cytonuclear ratios show that epidermal cells at the micropylar end of the cotton ovule are more highly differentiated than at the chalazal end. Ovules of *Pancratium maritimum*, which live along the Mediterranean coast, are protected from the entrance of seawater and from dessication through the micropyle by a compact layer of cells derived from the inner integument and nucellus
after fertilization (Werker and Fahn, 1975). During germination, the micropyle has been shown to be the principal pathway for the influx of water (Preston and Scott, 1943; Kyle, 1959) and for the exchange of gases (Wager, 1974).

**Nucellus**

The nucellus, defined by Heslop-Harrison (1972) as the wall of the megasporangium, is classified according to the position of the MMC relative to the apical nucellar epidermis. In ovules where the MMC is hypodermal, the nucellus is said to be tenuinucellate. The opposite condition, called crassinucellate, is where the MMC is found several cell layers beneath the epidermis. Pseudocrassinucellate ovules are somewhat intermediate with the MMC being initially hypodermal but subsequently becoming several cells deep due to proliferation of the nucellus. According to Davis (1966), out of 314 families surveyed, 179 are crassinucellar, 105 tenuinucellar, and 11 are pseudocrassinucellar. Among 19 families, including the Liliaceae, this character is generic. *Ornithogalum gussonei* and *O. umbellatum* have been identified as crassinucellar (Czapik, 1972).

The nucellus is generally the first tissue of an ovule to be initiated, and where an ovule is initiated along the placenta seems to be of great importance in regard to the fertilization of some taxa. In *Pelargonium* where there are two ovules per ovary, the upper ovule is preferentially fertilized (Tilney-Bassett, 1965; Tsai et al., 1973). In *Lupinus*, seed set is highest in the second ovule position from the peduncular end and decreases toward the stylar end of the ovary (Horovitz et al., 1976).
Horovitz et al. also demonstrated that there is a highly significant difference in seed set between the two valves of *Medicago sativa* and in some wild species of *Medicago* as well. It varies between species as to whether the right or left valve is more fertile, but the even-numbered ovule positions generally have the highest percentage of fertilization.

The nucellus is usually clearly outlined from the level where the integuments originate. From this point toward the micropyle, the nucellus and integuments each have their own distinct epidermal layers. In the opposite direction, toward the chalaza, the nucellus and integuments are confluent with the funiculus. The region where all sporophytic parts of the ovule merge with one another is known as the chalaza (Esau, 1965). See also Dahlgren (1927).

Pacini et al. (1975) divided the nucellus of *Diplotaxis* into two parts. The longer one of the two consists of empty degenerating cells lying in direct contact with the antipodals. The shorter one has cells rich in cytoplasm and is in contact with the outer integument where the vascular bundle ends. Norstog (1974) recognized two nucellar cell types in *Hordeum*—irregular highly vacuolate interior cells which undergo lysis, and rectangular epidermal cells that persist through early embryogenesis. Cook (1903b) recognized two cell types in the nucellus of *Agrostemma* also. Thin walled cells form an inner zone around the megagametophyte and then degenerate allowing the megagametophyte to expand. The second cell type has thick walls and forms an outer zone of cells aligned in rectangular rows radiating from the inner zone. Many of the nucellar cells of *Diplotaxis* are multinucleate (Pacini et al., 1975) while in *Helianthus* (Newcomb, 1973a) nucellar nuclei have only a moderate amount of condensed
chromatin and small, nonprominent nucleoli. The cells are largely vacuolate with small numbers of organelles which suggested to Newcomb that the nucellus of Helianthus is not synthetically active.

Parts of the nucellus other than the reproductive tissue, i.e., the vegetative tissue, may become disorganized during development of the megagametophyte and embryo sac in order to keep pace with the growing reproductive tissue. The vegetative tissue may be partly or completely resorbed. Breakdown of the nucellus in Hordeum results in what Norstog (1974) termed the nucellar lysate. Norstog concluded that the cells undergo lysis vice mechanical destruction by pressure. Reed (1944) found that insufficient amounts of zinc enhance cell necrosis and lysis in Pisum ovules. Large nucelli may be partly retained, and in some plant groups they form perisperm, a storage tissue in seeds, e.g., Yucca (Horner and Arnott, 1966).

In most instances, however, the nutritive function of the nucellus is completed during the early stages of embryo development. Generally, lipids and starch are the most important nutrients stored in the nucellus, e.g., Agave (Tilton, 1974), Bothriochloa (Moskova, 1975), and Diplotaxis (Pacini et al., 1975), but in some, e.g., Euphorbia, protein bodies are the most important (Gori, 1976). The perisperm of Yucca contains both protein bodies and lipid bodies (Horner and Arnott, 1965).

Coe (1954), in the only study of its type, used $^{14}$C to follow nutrient movement in the ovules of Zephyranthes drummondii. Coe found the greatest concentration of soluble assimilates in the chalazal end of the ovule and in the megagametophyte. Insoluble assimilates are most abundant in the nucellus and antipodals. Maciejewska-Potapczyk et al. (1974), however,
found no differences in soluble sugars between the micropylar and chalazal ends of the ovules in *Iris*. Malik and Vermani (1975) attempted a histochemical analysis of nutrients and enzymes in the ovules of *Zephyranthes rosea* and *Lagenaria vulgaris*, but their illustrations are of a quality which makes their conclusions questionable. Uhl and Moore (1971) found that tannins commonly accumulate in the chalaza of many palms.

**Modifications** In a number of taxa, the chalaza and chalazal end of the nucellus become variously modified as the ovule develops. In several cases, there is an axially arranged group of cells in the chalaza which Westermaier (1890, 1897) called the "Zuleitungsbahn" (conducting passage) or "Stärkestrasse" (starch route), as found in *Tricyrtis* (Ikeda, 1902). Ikeda speculated that these cells are involved in nutrition and in synthesizing enzymes for lysing nucellar cells in *Capsella* form what Schulz and Jensen (1971) call the chalazal proliferating tissue. They believed that this tissue has a nutritive function as well.

**Hypostase** According to the definition of Van Tieghem (1869), a hypostase is any modification in the chalaza, and this is also the way in which Johansen (1928) used the term. Schnarf (1929), however, restricted the use of the term to the corky or lignified cells which appear in the early development of the ovule's chalazal region. Netolitzky (1926) referred to cells of this same region which appear later in development as chalazal cork tissue, but Schnarf (1929) states that it is difficult to distinguish between the two conditions. Schnarf's definition for the hypostase will be used in this paper.

Ovules in a wide variety of taxa develop a hypostase, and hypostases are of common occurrence. They may be found among members of the Liliaceae
(Fulvio and Cave, 1964), Polygonaceae (Neubauer, 1971), Agavaceae (Tilton, 1974), Cruciferae (Prasad, 1975), and Euphorbiaceae (Bor and Kapil, 1976), and many others (Maheshwari, 1950). Histochemical test results show that carbohydrate and protein materials accumulate in the vacuoles as well as in the cytoplasm of hypostase cells in Agave. Test results also show that the highest concentration of lipids within the ovule is found within the hypostase. In addition to this nutrient supply, the Agave hypostase has numerous plasmodesmata and mitochondria (Tilton, 1974). Tilton correlated these structural and physiological features with the fact that the hypostase is located between the terminus of the vascular tissue feeding the ovule and the megagametophyte, and he postulated a nutritive function for the hypostase.

**Postament and podium** In Quercus, the postament, a group of persistent nucellar cells which projects into the megagametophyte, contains stored starch (Brown and Mogensen, 1972). By the time most of the inner integument is absorbed by the developing megagametophyte, the postament has been absorbed also (Mogensen, 1973). Ultrastructurally, the postament and inner integument cells are very similar. Plasmodesmata are few but there are many mitochondria and ribosomes, and the ER is abundant. Most postament cells are uniform in size and shape except for a few elongated cells near the center. These central cells have dense cytoplasm containing numerous ribosomes, ER, and mitochondria, and stain intensely for nucleic acids (Mogensen, 1973). A postament is found in several other taxa also, e.g., Palmae (Robertson, 1976). Somewhat different, the podium, as described by Dahlgren (1940), is the remnant of a cuplike nucellar structure at the chalazal end of the megagametophyte.
Such podia are reported to occur in *Codiaeum variegatum* (Bor and Bouman, 1974) and *Euphorbia geniculata* (Bor and Kapil, 1975).

**Nucellar plasmodium**  In the chalazal nucellus of some Podostemaceae, there is a gradual dissolution of the cell walls with a subsequent fusion of the freed protoplast. This condition was first described by Went (1910, 1926) as a hollow space containing many large degenerating nuclei and he termed it a pseudo embryo sac. Jäger-Zürn (1967) and Arekal and Nagendran (1975a,b), however, contend that the free nuclei in the pseudo embryo sac are not degenerate. Arekal and Nagendran (1975a,b) suggest that this modification be renamed a nucellar plasmodium and contend that it functions in nutrition of the megagametophyte and/or embryo depending on the species. In Pandanus, nucellar nuclei are reported to migrate into the central cell of the megagametophyte (Cheah and Stone, 1975). Migrating nucellar nuclei also occur in several other taxa (Maheshwari, 1950).

**Nucellar extensions**  In many cases the micropylar end of the megagametophyte apparently destroys all of the nucellar tissue surrounding it and then protrudes to some degree into the micropyle (Coulter and Chamberlain, 1909). For example, following meiosis in *Parnassia*, the nucellus degenerates in the micropylar region leaving only the chalazal end of the megagametophyte enclosed by nucellar tissue. This directly exposes the megagametophyte to the micropyle (Pace, 1912). The subject of megagametophyte protrusion into the micropyle is included in the section on the egg apparatus.

**Nucellar cap**  In some ovules, however, the nucellar integument remains intact at megagametophyte maturity and forms a uniseriate
layer of cells around the micropylar end of the megagametophyte as in Chlorogalum and Schoenolirion (Cave, 1974). Occasionally, the nucellar integumentary cells directly over the apex of the megagametophyte are elongate and palisadelike as exemplified by Ornithopus (Wojciechowska, 1972a) and Agave (Tilton, 1974). These cells in Agave have proximally thickened radial walls which attenuate distally (Tilton, 1974).

In a different condition, apical cells of the nucellar epidermis elongate and divide periclinally one to several times forming a more distinct nucellar cap. Cell walls of the nucellar cap may be thickened or suberized (Maheshwari, 1950). Such nucellar caps occur in a wide variety of taxa, e.g., Cactaceae (Neumann, 1935) and Liliaceae (Cave, 1955; Fulvio and Cave, 1964; Sterling, 1973b,c, 1975). The nucellar cap in Acer saccharinum consists of cells in files of 8-12 cells each (Haskell and Postlethwait, 1971). In Stellaria media, the distal cells of the cap are prolonged as papillae which project into the micropyle, and the proximal cells are cytoplasmically dense and stain darkly (Gibbs, 1907). Also, in some grasses, e.g., Avena (Cannon, 1900), Stipa (Narayanaswami, 1956), Hordeum (Cass and Jensen, 1970), Agrostis, and others (Maze and Bohm, 1974), the nucellus projects into the micropyle and this projection is thought by Maze and Bohm (1974) to aid in pollen tube guidance.

**Nucellar beak** In yet another condition, particularly among some members of the Cucurbitaceae, Euphorbiaceae, Nyctaginaceae, Polygonaceae, and Salicaceae, the apical portion of the nucellus forms a characteristically beak-shaped extension which protrudes into the micropyle or beyond (Maheshwari, 1950). Selected examples include Euphorbia (Lyon, 1898), Polygonum (Engell, 1973), and avocado in which epidermal cells of
the nucellar beak become elongated (Tomer and Gottreich, 1976). The nucellar beaks of *Euphorbia* store large protein crystals (Gori, 1976), and in *Codiaeum* they store starch (Bor and Bouman, 1974) indicating a possible nutritive role.

Subdermal cells of the nucellar beak in *Euphorbia geniculata* (Bor and Kapil, 1975) degenerate into a mucilaginous mass, while in *E. corollata* (Lyon, 1898) an axial row of cells becomes larger and looser than the surrounding cells. This axial file develops through the entire length of the beak, and the cells composing it break down as a pollen tube approaches the ovule. Degeneration of nucellar tissue forming such a pathway for pollen tube growth is also reported to occur in *Agrostemma* (Cook, 1903b), *Gossypium* (Balls, 1905), *Beta* (Artschwager and Starrett, 1933), *Cardiospermum* (Kadry, 1946), and several others (Lloyd, 1899).

Among the Euphorbiaceae where obturator and nucellar beak development often are correlated closely (Schweiger, 1905), the nucellar beak may extend beyond the micropyle until it comes into contact with the obturator at which time it then forms a swollen tip, e.g., *Croton* (Landes, 1946). The record for nucellar extension is probably the report by Rauch (1936) for *Scurrula atropurpurea* and *Dendrophthoe pentandra* in which the nucellus tip grows to about half way up the style. In the Balanophoraceae, e.g., *Balanophora polyandra* (Hofmeister, 1859), the nucellus elongates to form a stylelike structure, complete with a canal down which pollen tubes grow. Pollen thus comes into direct contact with the nucellus as it does in gymnosperms. *Balanophora polyandra* also has megasporangia which resemble archegonia (Coulter and Chamberlain, 1909).
Funiculus

Ovule vascular supply In most instances, the ovule is fed by a single vascular strand which traverses the funiculus and terminates at the chalaza. Among palms, an individual ovule may be supplied by anywhere from one to as many as fifteen vascular bundles, depending on the species (Uhl and Moore, 1971). In some instances, e.g., Chrozophora (Bor and Kapil, 1976), the funicular vascular bundle has been shown to be collateral, and, in Dianthus (Buell, 1952b), by the time of fertilization the funicular bundle contains a number of protoxylem and metaxylem elements. Also in Dianthus, a number of elongated nucellar cells extend from the funiculus toward the chalazal end of the ovule. These cells are thought by Buell (1952a,b) to act as a supplementary conducting tissue within the ovule.

Penetration of vascular tissue into the nucellus is not common, but it has been reported to occur in Agave lechuguilla (Grove, 1941), Acalypha rhomboidea, and a few others (Landes, 1946). In Castanea (Benson, 1894), Asclepias (Frye, 1902), and Carpinus (Benson et al., 1906), trachleds are present in the nucellus, but they are not connected with the funicular strand as they are in some Capparidaceae and Resedaceae (Orr, 1921a,b). In Strombosia (Fagerlind, 1947), vascular tissue extends up to the megagametophyte whereas that of Acalypha (Landes, 1946) extends only to the hypostase where it then branches and penetrates into the nucellus proper for a short distance. Swamy (1948) claimed that the funicular bundle of Casuarina extends up to the sporogeneous tissue and that some of the sporogeneous cells then elongate and become conducting cells rather than megaspores. This latter report merits reinvestigation.
In a few instances, vascular tissue is found in the integuments. If two integuments are present, vascular tissue may be found in both or only in the outer (Esau, 1965). The latter condition is found in Quercus (Brown and Mogensen, 1972; Mogensen, 1973) and in Jubaeopsis, a palm (Robertson, 1976). In Magnolia, the main vascular bundle bifurcates near the chalaza. One branch extends into the hypostase where it becomes surrounded by tanniniferous cells, and the other branch divides once more into strands which penetrate the outer integument (Boer and Bouman, 1972). An exception to the general rule of vascular tissue not being present in the inner integument is Euphorbia geniculata (Bor and Kapil, 1975).

Among the extant literature on ovules, there are only two papers which deal exclusively with the funicular vascular bundle supply, i.e., Monnier (1872) and Kühn (1928), and only one paper is devoted specifically to vasculature in seeds, i.e., Netolitzky (1926). To date, the most thorough coverage of vascular tissue extending into the nucellus is the treatment by Landes (1946).

Ovule attachment and curvature. In addition to its role in providing a pathway through which vascular tissue passes, the funiculus is the structure by which most ovules are attached to the placenta. Among palms, however, Jubaeopsis caffra (Robertson, 1976) is an interesting exception in that there is no funiculus and the ovule proper is affixed to the placenta directly. According to Laubengayer (1937), some members of the Polygonaceae are also without a funiculus. Laubengayer believed that the stalk of the ovule in single-ovule members of the Polygonaceae was derived by a reduction in the many-ovule forms. He concluded that
the supporting structure in the single-ovule forms thus represents a reduced free central placenta. Joshi (1938) refuted Laubengayer and claimed the presence of a true funiculus.

Most funiculi are unbranched, but branching does occur among some members of the Orchidaceae (Abe, 1972) as well as in the Cactaceae and in the Genus *Adansonia* (Heel, 1974). Heel considered this branching of the funicule to be a way in which seed yield could be increased.

Funicules are also in part responsible for the presence or absence of ovule curvature. Davis (1966) surveyed 315 families and found that 204 have ovules which are termed anatropous, a term introduced by Mirbel (1829) and first authoritatively defined by Schleiden (1837). Twenty families have orthotropous ovules and thirteen have hemianatropous ovules. Campylotropous ovules occur in five families, amphitropous in four, and anacampylotropous and circinotropous ovules occur in one family each. Ovule curvature among the remaining families is a generic character (Davis, 1966). In pineapple, for example, most of the ovules are anatropous but three percent are orthotropous. In this particular case, the orthotropous ovules develop only partially and then abort (Wee and Rao, 1974).

Anatropous ovules usually originate as orthotropous ovules, but early on develop a curvature, e.g., some Cruciferae where ovule curvature is concurrent with MMC differentiation (Prasad, 1975). Curvature most commonly occurs at the same level as the origin of the inner, or only (if unitegmic) integument. As the integuments grow, curvature increases and usually before the outer integument is complete the nucellus is inverted against the funiculus. For this reason, anatropous ovules
with two integuments generally do not have an outer integument on their funicular side (Coulter and Chamberlain, 1909).

**Outgrowths** The funiculus also may give rise to some extra-funicular tissue as in the case of *Tieghemopanax sambucifolius* (Rao, 1972), among others, where it produces an obturator. The funiculus of several Rubiaceae produces a strophiole (Lloyd, 1899), in *Pancrotium parviflorum* it produces an elaiosome (Werker and Fahn, 1975), and in *Strelitzia* it contributes to the tuft of bright orange hairs surrounding the micropyle in that genus (Venter, 1976). Among cacti, the funicular covering of Opuntieae is commonly called an aril or a third integument, but it has been shown to be different from an aril and to be a true funicular covering (Flores, 1976; Flores and Engleman, 1976). In *Aloe*, however, a third integument is present as an aril and is produced by the funiculus (McNaughton and Robertson, 1974).

**Central cell**

The central cell is the largest cell of the megagametophyte and lies between the antipodals at its chalazal end and the egg apparatus at its micropylar end. The central cell is binucleate, its nuclei frequently being the largest in the megagametophyte (Jensen, 1973). Usually these polar nuclei, so called because of their origin at opposite poles of the megagametophyte, are so similar that once they have come together it is difficult to distinguish one from the other. When there is a difference in size between the two, it is usually the micropylar one which is larger (Maheshwari, 1950).
Polar nuclei  The polar nuclei of most taxa migrate toward the center of the cell from their respective poles to form the fusion (secondary) nucleus. The degree of fusion at the time of fertilization varies with the taxon and may be completed as in Agave (Regen, 1941; Mogensen, 1970) and Helianthus (Newcomb, 1973a,b) or only partly fused as in Gossypium (Gore, 1932; Jensen, 1963, 1965b; Jensen and Fisher, 1967b), Zea (Diboll and Larson, 1966; Diboll, 1968), Epidendrum (Cocucci and Jensen, 1969a), Hordeum (Cass and Jensen, 1970), and Petunia (Van Went, 1970b,c). The polar nuclei do not fuse prior to fertilization in Barleria and Crabbea (Karlström, 1974a) and are reported never to unite in Statice japonica (Ya and Masa, 1941), some species of Tulipa (Pechenitsyn, 1972), Melampyrum and Parentucellia (Greilhuber, 1973). In the latter three examples, only the micropylar polar nucleus participates in double fertilization resulting in 2n endosperm. In each case, endosperm ploidy level was determined with karyotypes to substantiate the claim.

The secondary nucleus most commonly occupies a position near the egg, often touching it in Senecio aureus (Mottier, 1893) and some Alsinoidaeae (Gibbs, 1907). In those plants where the secondary nucleus occupies a chalazal position, as characteristic for taxa with Helobial type endosperm (Schnarf and Wunderlich, 1939), e.g., Agave (Tilton, 1974), or where it occupies a central position, e.g., Hordeum (Cass and Jensen, 1970), Helianthus (Teleżyńska and Teleżyński, 1973), and many others (Maheshwari, 1950), it is connected to the egg apparatus by a cylindrical strand of cytoplasm as first described in Crocus by Hofmeister (1857). In Triticum, both polar nuclei have their own cytoplasmic strand, each of which connects with the antipodals and with the egg (Alexandrov and
Alexandrova, 1944). Histochemical analysis of the cytoplasmic strand in Agave shows it to be highly proteinaceous (Tilton, 1974). The polar nuclei and egg apparatus of cotton (Gore, 1932) and Capsella (Schulz and Jensen, 1973) are joined by a mass of cytoplasm rather than by a discrete strand, and in Senecio (Mottier, 1893) the secondary nucleus is suspended by a network of delicate cytoplasmic threads radiating from the egg apparatus. Ikeda (1902) postulated that such cytoplasmic strands provide the means by which the antipodals maintain communication with the egg apparatus, and that it provides the vehicle by which the polar nuclei can unite.

Just as the polar nuclei, the polar nucleoli are also quite large (Jensen, 1965b; Vazart and Vazart, 1965; Diboll and Larson, 1966; Cass and Jensen, 1970; Schulz and Jensen, 1973; Newcomb, 1973a). Nucleolar vacuoles are reported to occur in Zea (Diboll and Larson, 1966), Hordeum (Cass and Jensen, 1970), Capsella (Schulz and Jensen, 1973), Jasione (Erdelská, 1973), and Helianthus (Newcomb, 1973a); and in Capsella (Schulz and Jensen, 1973) the nucleolar periphery is mainly granular but contains fibrillar material as well. In Gossypium (Jensen, 1965b), Petunia (Van Went, 1970b), and Capsella (Schulz and Jensen, 1973), the cytoplasm between the two polar nuclei prior to fusion was noted to contain various organelles. Jensen (1965b) reported the presence of large nuclear ribosomes in the polar nuclei of Gossypium, and micronucleoli have been reported in Hordeum (Cass and Jensen, 1970) and Capsella (Schulz and Jensen, 1973). The occurrence of micronucleoli in Gossypium is controversial as they are reported not to occur (Jensen, 1965b) and to occur singly by the same author (Jensen, 1973).
The nuclear membranes of the polar nuclei are highly porous in 
\textit{Gossypium} (Jensen, 1965b), \textit{Capsella} (Schulz and Jensen, 1973), and \textit{Helianthus} (Newcomb, 1973a). Some connections were noted between the nuclear membranes and the ER in \textit{Gossypium} (Jensen, 1965b) and \textit{Capsella} (Schulz and Jensen, 1973). The nuclear envelopes of \textit{Petunia} are poorly developed but sheets of ER connect the outer membranes of the two nuclei in some cases (Van Went, 1970b). Jensen (1965b) noted that in \textit{Gossypium} there are small projections of the inner membrane into swellings of the outer membrane. These projections are complex and contain within them membrane-bound structures. Their function is unknown.

\textbf{Cytoplasmic features} In most unfertilized ovules the central cell is highly vacuolate, but soon after the polar nuclei unite in \textit{Tricyrtis} (Ikeda, 1902) the central cell begins to fill with cytoplasm. Generally, cytoplasm is found around polar nuclei or the fusion nucleus in a thin layer, and around the cell periphery in varying degrees of thickness. In \textit{Helianthus}, the cytoplasm is thickest around wall ingrowths (discussed later) and the egg apparatus (Newcomb, 1973a), whereas in \textit{Capsella} there is a dense accumulation of cytoplasm near the egg apparatus (Schulz and Jensen, 1973). In \textit{Linum} (Vazart, 1969), the narrow canal joining the chalazal and micropylar cavities of the megagametophyte is filled with cytoplasm. The large vacuole of the central cell has been postulated to serve as the storage site for carbohydrates, amino acids, and inorganic salts to be used later for embryo and endosperm nutrition (Ryczkowski, 1964; List and Steward, 1965). A large bundle of raphide crystals has been reported in the central cell vacuole of \textit{Aspidistra elatior} (Golaszewska, 1934).
Starch and/or lipids are commonly found in the central cell (Maheshwari, 1950; Buell, 1952b; Jensen, 1965b; Vazart and Vazart, 1965, 1966; Diboll and Larson, 1966; Diboll, 1968; Schulz and Jensen, 1968a, 1973; Van Went, 1970b; Cass, 1972; Prasad, 1974). Starch may occur in such large quantities as to obscure observations of the other central cell contents as in Arachis, Penstamon, and some others (Maheshwari, 1950). The stage at which starch makes its appearance in the megagametophyte varies in different plants and may occur as early as the MMC stage or not until shortly after fertilization, e.g., Dianthus (Buell, 1952b). The usual case is where starch makes its appearance when the megagametophyte is mature and reaches a maximum amount shortly after fertilization (Maheshwari, 1950).

Plastids are less numerous than mitochondria in the central cell of Petunia (Van Went, 1970b). They contain a small number of thylakoids and are filled with starch at maturity. In Helianthus (Newcomb, 1973a), they are undifferentiated except for small lamellae. In Capsella (Schulz and Jensen, 1973), central cell plastids are located primarily around the polar nuclei and primary endosperm nucleus with some in the lateral and chalazal cytoplasm as well. The internal plastid membranes are in stacks of three to five fused thylakoids, and the chloroplast matrix is filled with ribosomes and osmiophilic droplets. The presence and amount of starch in these plastids is variable and is probably dependent on the physiological conditions at the time of fixation. Plastids with central constrictions were frequently seen and Schulz and Jensen (1973) interpreted them as dividing. In Linum, the central cell contains many proplastids when the embryo is mature (Vazart and Vazart, 1966). Large
droplets that probably consist of lipid are numerous near the chalazal end of the egg apparatus in *Helianthus* (Newcomb, 1973a).

The central cell mitochondria of *Gossypium* are mostly large and may have one or more continuous cristae. There are also many smaller mitochondria found with portions of the outer membrane missing. Jensen (1965b) claims that these single membrane portions probably result from splitting of the mitochondria through continuous cristae. In *Petunia*, the mitochondria are moderate in number and vary in shape from oval to spherical with short, randomly distributed cristae (Van Went, 1970b). In *Zea* (Diboll and Larson, 1966), the mitochondrial profiles of the central cell resemble those of the antipodals. The large number of mitochondria found in the central cell of *Linum* are quite variable in size (Vazart and Vazart, 1965, 1966), while in *Hordeum* they are also numerous but are consistently small (Norstog, 1972b). The central cell cytoplasm of *Capsella* (Schulz and Jensen, 1973) contains numerous, randomly distributed spherical or oval mitochondria with short vesiculate cristae. The mitochondria contain ribosomes, intramitochondrial granules, and fibrils with DNA. Mitochondria partitioned by a single crista were interpreted as dividing. A single giant mitochondrion is close to the polar nuclei shortly before and after the time of fertilization.

Endoplasmic reticulum is abundant in the central cell cytoplasm of *Gossypium* (Jensen, 1965b), *Linum* (Vazart and Vazart, 1965, 1966), *Zea* (Diboll and Larson, 1966), *Epidendrum* (Cocucci and Jensen, 1969a), *Capsella* (Schulz and Jensen, 1973), and *Helianthus* (Newcomb, 1973a). While the RER of *Hordeum* (Norstog, 1972a) is more pronounced in the central cell than in the egg, that of *Petunia* (Van Went, 1970b) is poorly
developed and is found mostly near the plasma membrane and nuclear envelope. The RER in Capsella (Schulz and Jensen, 1973) is in long single profiles scattered throughout the cytoplasm but also frequently parallels the contours of the nucleus and various organelles, especially plastids. Short vesiculate pieces of ER closely parallel the plasmalemma where the central cell borders the egg, synergid, and integuments. In Linum (Vazart and Vazart, 1966), ER is in loose coils; in Helianthus (Newcomb, 1973a), it is found in circular patterns around the vacuoles; and, in Gossypium (Jensen, 1965b), it is often in whorls. The ER of Epidendrum (Cocucci and Jensen, 1969a) is described as being a smooth tubular type as it is also in Gossypium (Jensen, 1965b). Long, narrow crystals were found within the ER cisternae of Capsella (Schulz and Jensen, 1973). In Zea, ER is present throughout the central cell cytoplasm except in the area of the apical pocket (Diboll and Larson, 1966).

Ribosomes are commonly very numerous and are found free, bound to membranes, and aggregated into polysomes in Gossypium (Jensen, 1965b), Linum (Vazart and Vazart, 1966), Epidendrum (Cocucci and Jensen, 1969a), Capsella (Schulz and Jensen, 1973), and Agave (Tilton, 1974). Although ribosomes are also abundant in Petunia, they are all found as monosomes (Van Went, 1970b). In Gossypium (Jensen, 1965b), Capsella (Schulz and Jensen, 1973), and Agave (Tilton, 1974), ribosomes were found in plastids, and in Capsella those ribosomes immediately adjacent to lipid droplets often stain more intensely and are arranged in straight chains perpendicular to the lipid droplet. Ribosomes were the most abundant organelle found in the cytoplasmic strand traversing the central vacuole of Agave (Tilton, 1974). Microbodies occur throughout the central cell cytoplasm
of *Capsella* and are frequently associated with lipid droplets (Schulz and Jensen, 1973).

Dictyosomes of varying size and number occur in the central cell cytoplasm of *Gossypium* (Jensen, 1965b), *Linum* (Vazart and Vazart, 1965), *Zea* (Diboll and Larson, 1966), *Petunia* (Van Went, 1970b), *Capsella* (Schulz and Jensen, 1973), and *Agave* (Tilton, 1974). *Zea* (Diboll and Larson, 1966) and *Petunia* (Van Went, 1970b) have a moderate number which are randomly distributed while those of *Agave* (Tilton, 1974) are more frequent around the egg apparatus. Dictyosomes in *Petunia* either have 3-5 cisternae and resemble those of immature cells, or they have 1-2 short cisternae surrounded by vesicles. Sometimes only the vesicles are present (Van Went, 1970b). Dictyosomes found in the central cell of *Gossypium* (Jensen, 1965b) have 5-6 cisternae which produce a large number of vesicles. Schulz and Jensen (1973) found no apparent particular orientation of dictyosomes in relation to the central cell wall in *Capsella*, but they do appear active and have from 3-6 cisternae.

**Cell wall**  
The wall surrounding the central cell conforms to the shape of the antipodals at its chalazal end, the nucellus, integument, or integumentary tapetum along its lateral walls, and the egg apparatus at its micropylar end. The wall is variable in its thickness from one part of the sac to another. Against the nucellus, integuments, or integumentary tapetum it is usually thick, but in *Agave* (Tilton, 1974) the wall is thin laterally and thickens slightly adjacent to, but not surrounding, the egg apparatus. The wall surrounding the egg apparatus attenuates chalazally so that the central cell and egg apparatus are separated by only their respective plasmalemmas in *Gossypium* (Jensen,
1963, 1965a,b), Zea (Diboll and Larson, 1966; Diboll, 1968), Linum (Vazart and Vazart, 1965, 1966), Petunia (Van Went, 1970b), Quercus (Mogensen, 1972), Helianthus (Newcomb, 1973a), and Persea (Tomer and Gottreich, 1976). The cell wall is only partially complete around the egg apparatus of Agave (Tilton, 1974), and wall material is reported to occur between the central cell and egg apparatus of Capsella (Schulz and Jensen, 1973), Epidendrum (Cocucci and Jensen, 1969a), and Aquilegia (Vijayaraghaven et al., 1972).

Wall ingrowths of the type described by Gunning and Pate (1969) as transfer walls are not uncommonly found in the chalazal and/or micropylar regions of some central cells as in Linum (Vazart and Vazart, 1966; Vazart, 1968), Helianthus (Newcomb and Steeves, 1971; Newcomb, 1973a), Lobelia (Torosian, 1971), Eschscholtzia (Nagi, 1972), and Stellaria (Newcomb and Fowke, 1973). Although no reference is made by the author in the text, three illustrations show that wall projections occur in the micropylar end of the central cell of Gossypium, cf., fig. 4 Jensen 1963, fig. 6 Jensen, 1965b, and fig. 3 Jensen, 1968b. No such ingrowths occur in the mature megagametophyte of Capsella (Schulz and Jensen, 1973) or Pisum (Hardham, 1976), but they do form during early embryo development in these two taxa. The central cell of Aquilegia vulgaris produces haustoria which grow around the antipodals and into the chalazal nucellus (Rifot, 1973).

Anitpodals

Anatomy, cytology, behavior Antipodals are the most variable cells of the megagametophyte. About the only feature they share in
common is their location at the chalazal pole. Here they may be arranged all in the same horizontal plane as in Delphinium (Mottier, 1895), or they may be located slightly laterally as in some grasses (Maze et al., 1970), or, as in most cases, in a pocket formed by nucellar cells, e.g., Quercus (Brown and Mogensen, 1972). Vesque (1879) was of the opinion that antipodals are to be regarded as rudimentary useless and of value only in a morphological sense. Westermaier (1892), however, concluded from his examination of 34 unrelated species that antipodals are of considerable importance in the ultimate nutrition of the embryo. Westermaier's hypothesis was subsequently expanded and substantiated by Osterwalder (1898), Goldflus (1899), Lloyd (1899), Ikeda (1902), and several other authors, including some contemporary workers.

In many instances, antipodals are short-lived, sometimes degenerating prior to megagametophyte maturity, e.g., Claytonia (Cook, 1903a), Trianthema (Bhargava, 1935), Agave (Regen, 1941; Mogensen, 1972), Dianthus (Buell, 1952a), Gossypium (Gore, 1932; Jensen, 1963, 1965b), Ornithopus (Wojciechowska, 1972a), Pseuderanthemum tunicatum (Karlström, 1973), Euphorbia (Bor and Kapil, 1975), and Jubaeopsis (Robertson, 1976). In pineapple, the antipodals degenerate 12-24 hours after anthesis (Wee and Rao, 1974), but in Senecio (Mottier, 1893), Decaisnea (Swamy, 1953), Paeonia (Cave et al., 1961), Epidendrum (Cocucci and Jensen, 1969b), and Pseuderanthemum alatum and P. tuberculatum (Karlström, 1973) they persist until embryo development is well underway. In many grasses (Westermaier, 1890), some Acanthaceae (Karlström, 1973), and in 47 other families (Davis, 1966) the antipodals not only persist, but they may also proliferate. In Vaillantia and Galium (Lloyd, 1899), two antipodals
degenerate and the third enlarges greatly and subsequently fills with cytoplasm.

The number of antipodals in the mature megagametophyte ranges from one in some Rubiaceae (Lloyd, 1899), two among many composites (Newcomb, 1973a; Teleżyńska and Teleżyński, 1973; Howe, 1975), four in Potamogeton (Wiegand, 1900), and Linum (Vazart, 1969), and following fertilization up to as many as 150 in Sparganium (Campbell, 1899) and several hundred in Sasa paniculata, a bamboo (Yamaura, 1933). Campbell (1899) concluded that the ultimate size and numerical increase of antipodals is ultimately dependent upon fertilization.

The occurrence of a multiplicity of nuclei within antipodals is a common feature among many genera of eight families, with 2-3 nuclei/cell the most prevalent condition (Davis, 1966). Examples of taxa demonstrating a binucleate condition include Senecio (Mottier, 1893), Leucocrinum (Cave, 1948), and Helianthus (Newcomb, 1973a; Teleżyńska and Teleżyński, 1973) while Androcymbium (Cave, 1967) and Bellis (Engell and Petersen, 1977) may be 2-4 nucleate. The number of nuclei ranges from 3-4 in the antipodals of Agrostis (Maze and Bohm, 1974) and from 1-5 in Veratrum where the number may vary among the antipodals of a given individual as well (Sokolowska-Kulczycka, 1973). The record for the most nuclei seems to be 12, found in both Hepatica acutibola (Mottier, 1895) and Polygala sibirica (Davis, 1966). Some antipodal nuclei may become polyploid via endomitosis, e.g., Ornithogalum nutans and O. nanum (Karagozova and Van Khankh, 1972), and endopolyploid nuclei of 48 n have been reported for Chrysanthemum (Titz, 1965) and of 64 n for Crocus (Turala, 1966).
Antipodals may remain relatively small and inconspicuous as in *Quercus* (Brown and Mogensen, 1972), but they more commonly are relatively large cells. In some cases they may become extremely large as in *Aquilegia canadensis* (Mottier, 1895) where they appear to be some fifteenfold larger than the 4-celled embryo, and, in *Jeffersonia diphyllea* (Andrews, 1895) and *Chloris gayana* (Chikkannaiah and Mahalingappa, 1975), they occupy more than one half the megagametophyte's volume. And even larger, the antipodals of *Callipeltis* and *Sherardia* (Lloyd, 1899) extend three-fourths the length of the megagametophyte. The antipodals of *Tricyrtis* (Ikeda, 1902) are larger than the egg apparatus by the time of pollination and attain their maximum size just prior to fertilization, whereas those of *Aquilegia canadensis* (Mottier, 1895), *Sparganium* (Campbell, 1899), and *Hordeum jubatum* (Brink and Cooper, 1944) continue enlarging after fertilization. According to Chiappini (1962), the antipodals of *Ornithogalum* exhibit hypertrophic growth but do not proliferate.

Muniyamma (1976) postulated a hormone imbalance as the cause of multinucleate antipodals in *Agrostis pilosula*, while Diboll and Larson (1966) attribute this condition in *Zea* to incomplete wall formation. In *Helianthus* some freely growing antipodal walls were found extending across the central cell, apparently being deposited by the dictyosomes found at the growing cell wall tips (Newcomb, 1973a). Plasmodesmata are present in the walls between antipodals in *Capsella* (Schulz and Jensen, 1971) and in *Helianthus* (Newcomb, 1973a). They are also found between antipodals and central cell in both plants. Newcomb (1973a) found additional plasmodesmata between the antipodals and the integument in *Helianthus*, but these occur less frequently than do those between antipodals and central
cell. It has been noted that plasmodesmata do not occur between antipodals and nucellus in *Stipa* (Maze and Lin, 1975).

Pritchard (1964) reported that azure B bromide staining in *Stellaria* antipodals revealed low RNA content in these cells, but that intense Feulgen reactions indicated a high DNA content which he interpreted as resulting from endomitosis. He also noted the presence of histones within the nuclei. In *Helianthus*, Newcomb (1973a) demonstrated a sharp increase in toluidine blue staining as the antipodals degenerate, but he made no distinction between DNA and RNA. According to Heslop-Harrison (1972), however, antipodals become basiphilic indicating accumulation of rRNA. In *Hordeum* (Cass and Jensen, 1970), the 100 or so antipodals have PAS positive walls and nucleoli that stain darkly with aniline blue black. The antipodals of *Bellis* (Engell and Petersen, 1977) also have a high protein content, and lipids are found in the antipodals of *Zea* (Diboll and Larson, 1966) and *Stipa elmeri* (Maze and Lin, 1975). Starch is absent from the antipodals of *Hordeum* (Cass and Jensen, 1970), *Stellaria* (Pritchard, 1964), and *Agave* (Tilton, 1974), but starch grains are present in some members of the Rubiaceae (Lloyd, 1899), *Tricyrtis* (Ikeda, 1902), and in *Zea* (Diboll and Larson, 1966) among many others. The antipodals of *Ornithogalum* stain more intensely than do other cells of the megagametophyte (Raciborski, 1893b; Żabińska, 1972).

**Function** The presence of only few mitochondria and plastids, sparse ER, and the absence of dictyosomes in the antipodal cytoplasm suggested to Schulz and Jensen (1971) that the antipodals of *Capsella* are not highly synthetic. Schulz and Jensen also suggested that the presence of wall projections on the chalazal megagametophyte wall outside
the antipodals could be interpreted to mean that metabolites from the crushed nucellus may be directly absorbed by the central cell without first being metabolized by the antipodals. Conversely, the number of organelles, i.e., mitochondria, ribosomes, and RER, is high in the antipodal cytoplasm of Zea (Diboll and Larson, 1966; Diboll, 1968), Helianthus (Newcomb, 1973a), and Aquilegia (Rifot, 1973). Diboll (1968) also makes note that the dictyosomes of Zea antipodals are active in producing many large clear vesicles. Maze and Lin (1975) concluded that the antipodals of Stipa elmeri are the most cytologically active cells of the megagametophyte and that they have some characteristics in common with transfer cells which may indicate a role in the transferal of material into the megagametophyte.

The antipodal walls in Linum are described as having multiple folds and fingerlike projections which form the counterpart of the filiform apparatus of the synergids. It was suggested by Vazart (1968) that these structures form a barrier between gametophyte and sporophyte disallowing an intense transit of nutrients between the two generations. In antipodals, such wall ingrowths were first described in Zea by Diboll and Larson (1966) who, basing their argument on two papers by Schnepf (1963, 1964), concluded that these ingrowths have a function in material transfer into the central cell, and thus agree with Westermaler (1892) and a host of others that the antipodals have a nutritive role. Transfer wall ingrowths have also been reported to occur in Aquilegia (Rifot, 1973) and in Bellis (Engell and Petersen, 1977).

According to Coulter and Chamberlain (1909), there are two general types of persistent antipodals—passive and aggressive. In the passive
type, antipodals remain active, often become very much enlarged or even form a mass of tissue, but they are not associated with an invasion of the chalazal region. They simply receive material passively from the chalaza and nucellus. This type is characteristic for many monocots, except grasses. In the aggressive type of antipodal, active and often proliferating antipodals are associated with the penetration of the chalazal region by the antipodal portion of the megagametophyte. This type is characteristic of the Sympetalae, especially the Rubiaceae and Compositae, and is also characteristic of the Poaceae and Amentiferae. Among the Amentiferae, often both an antipodal haustorium and an endosperm haustorium are present.

Except for Vesque (1879) who believed that the antipodals have no physiological function, the principal authors of the late 1800's and most authors of the 1900's support Westermaier's hypothesis that antipodals have a significant role in megagametophyte and embryo nutrition. D'Hubert (1896), on the basis of the appearance and disappearance of starch, concluded that the antipodals nourish the megagametophyte prior to fertilization, and Goldflus (1899) interpreted antipodals as representing "...l'intermédiaire entre le sac embryonnaire et les substances digestibles élaborées par l'ovule." Ikeda (1902) concluded, "...the chromatin-aggregation in the nuclei of antipodals of Tricyrtis is also the expression of their metabolic activity, that therefore these organs play a most essential role in the nutrition of the embryo sac, --that they are indeed the metabolic centre for the absorption, elaboration, and transportation of nutritive materials of the latter." Ikeda also stated that the nutritive function of the antipodals continues from
megagametophyte maturation until endosperm formation. Cook (1903a) stated that the nucellus of *Claytonia* is absorbed into the megagametophyte through the antipodals, and Gore (1932) also concluded that conduction of nutrients into the megagametophyte of cotton is through its chalazal end.

Gibbs (1907) postulated that the enzymes which digest the nucellus during megagametophyte growth are derived directly from the megagametophyte. He concluded, "The chalaza is the seat of elaboration of proteoid material, and the whole of organized food supplies required from growth of the ovule and embryo, together with water and air must pass through this tissue."

In addition to their direct role in megagametophyte nutrition, Westermaier (1890) believed that the antipodals of *Zea* and other grasses function as endosperm cells until the true endosperm is formed. Brink and Cooper (1944) modified this postulate slightly and stated that in grasses the antipodals are involved in the control of endosperm development, and Maze and Lin (1975) speculated that the antipodals may thus be the site of growth controlling hormone synthesis.

**Egg apparatus**

**Egg** Typically the egg apparatus consists of one egg and two synergids and usually has completed differentiation before the antipodals (Maheshwari, 1941). Although generally smaller than the synergids, the egg of *Tricyrtis* (Ikeda, 1902), *Claytonia* (Cook, 1903a), and *Stipa* (Maze and Lin, 1975) is the largest cell of the egg apparatus. In the egg, the nucleus and most of the cytoplasm lie in the chalazal end of the
cell while the micropylar end is occupied by a large vacuole. This vacuole commonly accounts for the greater volume of the egg than do the nucleus and cytoplasm. In some eggs, however, the vacuole may be only about one-half the size of the egg, or the egg may contain only several small vacuoles, e.g., Capsella (Schulz and Jensen, 1968b) and Epidendrum (Cocucci and Jensen, 1969a). In Zea (Diboll and Larson, 1966), the egg has only a few small vacuoles near the micropyle and, thus, has more cytoplasm relative to most other eggs. The egg of Plumbago is a cell in which both gametic and synergid functions occur, and it is divided into three regions by Cass and Karas (1974): 1) lateral cytoplasm, 2) chalazal cytoplasm and nucleus, and 3) filiform apparatus and associated cytoplasm.

Eggs generally appear to be attached to only a limited region of the embryo sac wall, and in some species the egg may sit atop the synergids, e.g., Agave lechuguilla (Grove, 1941), or to their side as in Acer saccharinum (Haskell and Postlethwait, 1971). Except for Potamogeton (Wiegand, 1900) where no walls exist between cells of the egg apparatus, all eggs have a common surface with the synergids on one side, the micropylar side, and with the central cell on the chalazal side. Favre-Duchartre (1971, 1975) claims that this marginal position of the egg is what allows the egg to form a zygote while its sister cell in double fertilization, the central cell, forms endosperm.

The cell wall of the egg is generally thickest in the micropylar end and usually attenuates chalazally, commonly being absent from the chalazal half of the egg. This pattern has been known for a long time and includes Hepatica (Mottier, 1895), Stellaria (Gibbs, 1907), Gossypium
(Jensen, 1965a,b), Torrenia (Pluijm, 1964), Linum (Vazart and Vazart, 1966), Crepis (Godineau, 1966, 1969), Picris, Cichorium, and Calendula (Godineau, 1969), Petunia (Van Went, 1970b), Quercus (Møsgensen, 1972), Helianthus (Newcomb, 1973a; Telezynska and Telezynski, 1973), Oenothera (Jalouzot, 1975), and Stipa (Maze and Lin, 1975). There are a few exceptions in which wall material is reported to occur in the chalazal end of the egg. In Capsella (Schulz and Jensen, 1968b), Epidendrum (Cocucci and Jensen, 1969a), Plumbago (Cass and Jensen, 1974), and Agave (Tilton, 1974), it is not a complete wall in that periodic discontinuities exist. In Bellis, however, the wall does attenuate chalazally but it is entire (Engell and Petersen, 1977).

Plasmodesmata are present in the common walls between egg and synergid, and egg and central cell, but not between egg and sporophyte in Capsella (Schulz and Jensen, 1968b) and Agave (Tilton, 1974). In Helianthus (Newcomb, 1973a), a middle lamella is absent in all common walls of the egg apparatus, and in Agave (Tilton, 1974) a middle lamella is found only between the two halves of the filiform apparatus. Plumbago has a unique feature in that there are no synergids and the egg cell wall has PAS-positive ingrowths in the micropylar area which are interpreted as being the filiform apparatus (Cass, 1972; Cass and Karas, 1974).

Although generally smaller than either the polar nuclei or the fusion nucleus, the egg nucleus is characteristically large and has a single large nucleolus, e.g., Senecio (Mottier, 1893), Sparganium (Campbell, 1899) and Lycopersicon (Cooper, 1931). Nucleolar vacuoles have been reported in Stellaria (Pritchard, 1964), Hordeum (Cass and Jensen, 1970), Jasione (Erdelská, 1973), and Helianthus (Newcomb, 1973a),
and micronucleoli occur in *Hordeum* (Cass and Jensen, 1970). The egg nucleolus of *Capsella* (Schulz and Jensen, 1968b) is composed of two regions, a larger interior region composed of loosely packed amorphous material interwoven with fibrous material and an exterior region of dense, tightly packed granules. This type of nucleolar morphology is peculiar to the egg of *Capsella*. In *Gossypium* (Jensen, 1965b) and *Capsella* (Schulz and Jensen, 1968b), some connections between the nuclear membrane and ER were seen, but in neither did organelles form de novo from nuclear membrane evaginations as reported to occur in fern eggs (Bell and Mühlethaler, 1964). In both *Stellaria* (Pritchard, 1964) and *Gossypium* (Jensen, 1965b), the egg nucleolus shows high RNA and protein content, but DNA content is low in *Gossypium* (Jensen, 1965b) and shows no reaction in *Stellaria* (Pritchard, 1964). For more on the occurrence of basic proteins in relation to chromatin and their function in fertilization, see Dryanovska and Shahanova (1976).

The plastids of egg cells vary in shape from circular in *Zea* (Diboll and Larson, 1966) to rod-shaped or with cup-shaped invaginations in *Capsella* (Schulz and Jensen, 1968b). Their size and shape within a given egg may be relatively uniform as in *Gossypium* (Jensen, 1965b) or they may be of various shapes as in *Petunia* (Van Went, 1970b) and *Helianthus* (Newcomb, 1973a). Plastids in the egg of *Gossypium* (Jensen, 1965b), *Capsella* (Schulz and Jensen, 1968b), *Petunia* (Van Went, 1970b), and *Helianthus* (Newcomb, 1973a) are reported to contain lamellae. Starch is not found in the egg of *Plumbago* (Cass, 1972) or *Agave* (Tilton, 1974), but does occur in *Tricyrtis* (Ikeda, 1902), *Stellaria* (Pritchard, 1964) *Linum* (Vazart and Vazart, 1965, 1966; Vazart, 1969), *Gossypium* (Jensen,
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Endoplasmic reticulum is sparse in the egg of *Linum* (Vazart and Vazart, 1965), *Zea* (Diboll, 1968), *Petunia* (Van Went, 1970b), *Capsella* (Schulz and Jensen, 1968b), and *Helianthus* (Newcomb, 1973a). A unique type of ER occurs in *Gossypium* (Jensen, 1963, 1965b, 1968a). This ER contains tubes that are 29-31 nm in diameter, several μm in length, and are crooked or branched. The tubes run generally parallel to the ER which contains them, and they are open to the ground cytoplasm. These tubes are also found within the nuclear membrane. The function of these tubes is postulated to be part of the mechanism whereby the cell volume is reduced approximately fifty percent during formation of the zygote. In *Quercus* (Mogensen, 1972), there are abundant quantities of RER in the egg.

The number of mitochondria is high in the egg of all species studied thus far except for *Petunia* (Van Went, 1970b) and *Agave* (Tilton, 1974). They are usually spherical and/or rod-shaped as in *Zea* (Diboll and Larson, 1966), *Capsella* (Schulz and Jensen, 1968b), *Gossypium* (Jensen, 1965b), *Petunia* (Van Went, 1970b), and *Helianthus* (1973a). They also may be elongate as in *Zea* (Diboll and Larson, 1966) or oval as in *Petunia*. 
(Van Went, 1970b). Cristae are generally short and vesiculate and range from few in number in *Gossypium* (Jensen, 1965b), *Capsella* (Schulz and Jensen, 1968b), and *Helianthus* (Newcomb, 1973a) to many in *Petunia* (Van Went, 1970b) and *Quercus* (Mogensen, 1972). In both *Zea* (Diboll and Larson, 1966) and *Capsella* (Schulz and Jensen, 1968b), following fixation with osmium tetroxide, mitochondria appear very similar to plastids. Mitochondria in the egg of *Plumbago* are most numerous around the filiform apparatus, and the *Plumbago* egg in general is more metabolically active than in other species (Cass and Karas, 1974).

Dictyosomes are consistently few in number in eggs, and may be entirely lacking as in *Epidendrum* (Cocucci and Jensen, 1969a). The number of cisternae may range from 2-4 as in *Agave* (Tilton, 1974), 3-4 in *Gossypium* (Jensen, 1965b), 3-5 in *Petunia* (Van Went, 1970b), or 5-6 as in *Capsella* (Schulz and Jensen, 1968b). The dictyosomes of *Quercus* are of a noncompact type and have only a few associated vesicles.

Ribosomes of both the free and the membrane-associated types have been reported in *Gossypium* (Jensen, 1965b), *Linum* (Vazart and Vazart, 1965), *Capsella* (Schulz and Jensen, 1968b), *Quercus* (Mogensen, 1972), *Helianthus* (Newcomb, 1973a), and *Agave* (Tilton, 1974). Polysomes have been reported to occur in all the above plants except *Petunia* (Van Went, 1970b), *Quercus* (Mogensen, 1972), and *Agave* (Tilton, 1974). Some of the ribosomes in *Gossypium* occur in helical chains (Jensen, 1965b).

**Synergids** The remaining two cells of the egg apparatus, termed synergids by Strausberger (1879), are probably the most dynamic and structurally complex cells of the megagametophyte. Accordingly, Davis (1966) opined that synergid anatomy should be recorded only when the
megagametophyte is awaiting fertilization—unless qualified otherwise as to the stage of development. An exception to the typical three-celled egg apparatus is that found in the Plumbago type of megagametophyte developmental sequence in which no synergids develop (Maheshwari, 1950; Cass, 1972; Cass and Karas, 1974)

Not uncommonly, synergids are notched by an indentation resulting in the formation of a prominent hook toward the micropylar end of the cell. Dahlgren (1928) listed 52 species from 22 families as demonstrating this character. The Onagraceae and Compositae head Dahlgren's list with seven species each and the Labiatae is next with six. Individual examples include Houstonia (Lloyd, 1899), Bulbine (Stenar, 1928), Butomopsis (Johri, 1936), Ammannia (Smith and Herr, 1971), pineapple (Wee and Rao, 1974), Holarrhena (Lattoo, 1974), Chlorogalum, Schoenolirion (Cave, 1974), Agave (Tilton, 1974), Stypandra (Cave, 1975), some Poaceae (Chikkannaiah and Mahalingappa, 1975), and several Cruciferae (Prasad, 1975). As yet, there has been no functional significance attributed to this anatomical feature. The micropylar end of the synergids is also characterized by the presence of a filiform apparatus (discussed later) and by containing most of the cells' cytoplasm. The nucleus lies in or just chalazad the hook region, and the chalazal end of the cell is usually occupied by a large vacuole. An exception to the latter condition is found in Androcymbium (Cave, 1967) in which the synergids are reported to have no vacuoles.

Cell wall In Aquilegia (Vijayaraghaven et al., 1972), the synergid wall extends over the chalazal end of the cells, but where there is a common surface between egg and synergids and between the chalazal
end of sister synergids, the cells are separated by only their plasmalemmas. In Capsella (Schulz and Jensen, 1968a) and Epidendrum (Cocucci and Jensen, 1969a), cell wall material is found over the chalazal end of the cells and along the common surfaces of the egg apparatus. Some discontinuities are present in the common surfaces, however, so that in both plants there are regions where the cells of egg apparatus are separated by wall material and other regions where they are separated by only plasmalemmas. In Torenia (Pluijm, 1964), Gossypium (Jensen, 1965a), Linum (Vazart and Vazart, 1966; Vazart, 1969), Zea (Diboll and Larson, 1966), Quercus (Mogensen, 1972), Helianthus (Newcomb, 1973a), and Agave (Tilton, 1974), the synergid walls extend only over the micropylar one-third to one-half of the cell before it attenuates so that the remainder of the cell is enclosed by only its plasmalemma. Cell walls are absent entirely from the egg apparatus of Potamogeton (Wiegand, 1900).

Plasmodesmata are present in all walls of the synergids of Capsella (Schulz and Jensen, 1968a) and Agave (Tilton, 1974) except where the walls are common with the sporophyte. They are present between the synergids of Gossypium (Jensen, 1965a) and Helianthus (Newcomb, 1973a), but in the latter they disappear after the wall thickens. Plasmodesmata are rare in the synergids of Petunia (Van Went, 1970a) and absent in those of Quercus (Mogensen, 1972, 1973). A middle lamella is missing from the common walls of the egg apparatus of Helianthus (Newcomb, 1973a) and is present only between halves of the filiform apparatus in Agave (Tilton, 1974).

In Gossypium (Jensen, 1965a), the synergid walls stain positively for protein and carbohydrates. They contain two dense bands made up of
closely appressed cylinders having a diameter of 60 nm, these are thought by Jensen to be cellulose fibers or noncellulosic polysaccharides surrounded by a pectic sheath. The fibers run at right angles to the long axis of the cell. In the thicker part of the cell wall, the center is composed only of the fibers without the sheaths and thus appears to be clear. In Agave (Tilton, 1974), the synergid cell wall laterad the filiform apparatus is thick and fibrous, but thins as it approaches the hook region.

**Filiform apparatus** The filiform apparatus is a structure which characterizes mature synergids more than does any other feature. With the reported exceptions of Bomarea and Alstroemeria (Stenar, 1925; Cave, 1966), Ornithogalum gussonei (Desole, 1947), Magnolia stellata (Kapil and Bhandari, 1964), Farsetia hamiltonii, Brassica rapa, Malcolmia africana, Lepidium sativum, and Coronopus didymus (Prasad, 1975), and Erythrina caffra (McNaughton, 1976), the filiform apparatus seems to be universally occurrent. In that the filiform apparatus appears to be the last structure to develop in synergids, and, as noted by Davis (1966), require careful staining to demonstrate their presence. These few reports of no filiform apparatus are somewhat suspect and warrant reinvestigation.

Heslop-Harrison (1972) gives credit to Habermann (1906) for naming the filiform apparatus, but credit rightfully goes to Schacht (1857) who described and named it in Campanula, Watsonia, and Zea. In the same year, Hofmeister (1857) also described, but did not attempt to name, the filiform apparatus of Crocus. Filiform apparatus may be large and well-developed, e.g., Agave (Tilton, 1974), they may be small, e.g., Bellis
(Engell and Petersen, 1977), or they may be poorly developed, e.g., Hesperocallis and Leucocrinum (Cave, 1948) and Paeonia (Cave et al., 1961). Filiform apparati of various species are strongly refractive (Strausburger, 1879; Mottier, 1893), stain only slightly with alum cochineal (Mottier, 1893), stain intensely with Bismarck brown (Mottier, 1893) and PAS or other carbohydrate stains (Jensen, 1963, 1965a; Diboll and Larson, 1966; Van Went and Linskens, 1967; Schulz and Jensen, 1968a; Cass and Jensen, 1970; Van Went, 1970a; Vijayaraghaven et al., 1972; Cass, 1972; Mogensen, 1972; Tilton, 1974), and, in the living condition, filiform apparati stand out well when stained with Sudan III (Erdelská, 1968). Proteins occur in the filiform apparatus of Paspalum (Chao, 1971), Aquilegia (Vijayaraghaven et al., 1972), and Agave (Tilton, 1974), and pectins have been found in that of Gossypium (Jensen, 1965a).

Although Jensen (1963) was the first to describe filiform apparatus ultrastructure, Pluijm (1964) was the first to give a detailed account of its development, and according to him the apparatus forms from a thickening of the wall between synergids. This thickening is due to the deposition of material which is probably supplied by the many small nearby vacuoles. The plasmalemma between filiform apparatus and cytoplasm in Torenia is interrupted in many places by vacuoles voiding themselves in the direction of the apparatus. The wall between sister synergids meets the megagametophyte wall precisely at its micropylar apex and there forms an intercellular space. During filiform apparatus development, the megagametophyte wall above this cavity is partly dissolved and then bursts open (Pluijm, 1964). In Agrostis (Maze and Bohm, 1974), one synergid is said to develop a filiform apparatus in advance
Ultrastructurally, the filiform apparatus of *Torenia* has a homogeneous appearance (Pluijm, 1964) whereas in *Gossypium* (Jensen, 1965a), *Zea* (Diboll and Larson, 1966), *Petunia* (Van Went and Linskens, 1967; Van Went, 1970a), *Capsella* (Schulz and Jensen, 1968a), *Quercus* (Mogensen, 1972), *Plumbago* (Cass and Karas, 1974), and *Agave* (Tilton, 1974) it has been shown to have two phases which can be identified by differences in their electron translucence. Brown (1917) and Cooper (1931) noted density differences in the filiform apparatus of *Phaseolus* and *Lycopersicon*, respectively. Cooper (1931) also noted the occurrence of several elongate vacuoles which extend from the large chalazal vacuole to the striations of the filiform apparatus at the micropylar apex of the cell.

In *Gossypium*, the filiform apparatus have no common surfaces and are thus completely separate from the lateral walls of the synergids (Jensen, 1965a). In all other species examined to date, the apparatus share a common wall. The plasmalemma commonly follows the contour of the apparatus thereby increasing its surface area. In *Helianthus*, the apparatus is not filiform in shape, but does extend across most of the micropylar end of the cells (Newcomb, 1973a). Quite often the synergids and filiform apparatus are exposed directly to the micropyle as in *Bellis* (Engell and Petersen, 1977) and may even project into it as in *Lycopersicon* (Cooper, 1931) and *Forstera* and *Donatia* (Philipson and Philipson, 1973). Pace (1912) found in many instances that the entire egg apparatus of *Parnassia* protrudes into the micropyle. As the extreme example, Lloyd (1899) discovered that among several Rubiaceae the functional megaspore migrates into the micropyle following degeneration of the micropylar
nucellus. In the Genus *Asperula*, he commonly found thus developed ovules with the egg apparatus extending beyond the micropyle and pointing away from the funiculus.

**Cytoplasmic features** A single distinct nucleolus is found within the synergid nucleus of *Stellaria* (Pritchard, 1964), *Gossypium* (Jensen, 1963, 1965a), *Linum* (Vazart and Vazart; 1966; Vazart, 1969), *Zea* (Diboll and Larson, 1966), *Capsella* (Schulz and Jensen, 1968a), *Petunia* (Van Went, 1970a), *Hordeum* (Cass and Jensen, 1970), *Quercus* (Mogensen, 1972), *Helianthus* (Newcomb, 1973a), and, in *Agave* (Tilton, 1974), it may be lobed and have a large pars morpha phase. One or more micronucleoli are also found in *Gossypium* (Jensen, 1965a), and the pores of the nuclear envelop of *Quercus* appear to be plugged (Mogensen, 1972). The synergid nuclei of *Stellaria* (Pritchard, 1964), *Gossypium* (Jensen, 1965a), and *Quercus* had weak reactions for DNA.

The number of plastids varies from zero to few in *Petunia* (Van Went, 1970a) *Crepis*, *Picris*, *Cichorium*, and *Calendula* (Godineau, 1969), *Capsella* (Schulz and Jensen, 1968a), and *Quercus* (Mogensen, 1972), or many as in *Gossypium* (Jensen, 1965a), *Zea* (Diboll and Larson, 1966), *Linum* (Vazart and Vazart, 1969), and *Helianthus* (Newcomb, 1973a). Plastids are the most abundant organelle in the synergids of *Stipa* (Maze and Lin, 1975), and in *Agave* they occur in greater numbers in the synergids than in other cells of the megagametophyte (Tilton, 1974). Plastid distribution varies from being random in *Petunia* (Van Went, 1970a) to a micropylar-chalazal gradient in *Gossypium* (Jensen, 1965a), *Crepis*, *Picris*, *Cichorium*, and *Calendula* (Godineau, 1969). Plastids are slightly more numerous in the chalazal end of *Agave* synergids (Tilton, 1974). In *Capsella* (Schulz and
Jensen, 1968a), plastids are mainly in the area between the filiform apparatus and nucleus, and in Zea (Diboll and Larson, 1966) they are more or less evenly divided between poles.

Starch is not found in the synergid of Zea (Diboll and Larson, 1966), Petunia (Van Went, 1970a), Hordeum (Cass and Jensen, 1970), Aquilegia formosa (Vijayaraghaven et al., 1972), and Agave (Tilton, 1974). It is rare in Quercus (Mogensen, 1972); present in moderate amounts in Capsella (Schulz and Jensen, 1968a), Helianthus (Newcomb, 1973a), and Stipa (Maze and Lin, 1975); and occurs in large quantities in Gossypium (Jensen, 1965a), Aquilegia vulgaris (Fougère-Rifot, 1975), and Oenothera (Jalouzot, 1975). Large quantities of lipids are also found in the synergids of Aquilegia vulgaris (Fougère-Rifot, 1975) and Oenothera (Jalouzot, 1975).

Extensive amounts of ER, both smooth and rough types, are found in the synergids of Gossypium (Jensen, 1965a), Linum (Vazart and Vazart, 1966; Vazart, 1969), Petunia (Van Went and Linskens, 1967; Van Went, 1970a), Capsella (Schulz and Jensen, 1968a), Crepis, Picris, Cichorium, and Calendula (Godineau, 1969), Helianthus (Newcomb, 1973a), Agave (Tilton, 1974), Aquilegia (Fougère-Rifot, 1975), and Oenothera (Jalouzot, 1975). In Capsella (Schulz and Jensen, 1968a), some inorganic crystals are found within ER cisternae. Parallel orientation of the ER with the cell's long axis occurs in Gossypium (Jensen, 1965a), Petunia (Van Went and Linskens, 1967; Van Went, 1970a), Capsella (Schulz and Jensen, 1968a), and Aquilegia (Vijayaraghaven et al., 1972). In Agave, the longer segments tend to follow contours of the cell wall while shorter segments...
are at oblique or right angles to the wall. Short segments are often highly curved and are seen in L-, S-, and U-shaped configurations (Tilton, 1974).

Mitochondria are abundant in the synergids of all species studied to date. These mitochondria seem to be characterized by short vesiculate cristae except in Zea (Diboll and Larson, 1966) where the cristae are described as saccuolate, and in Petunia (Van Went and Linskens, 1967; Van Went, 1970a) Aquilegia (Vijayaraghaven et al., 1972), and Helianthus (Newcomb, 1973a) where they are tubular. In Gossypium (Jensen, 1965a), Capsella (Schulz and Jensen, 1968a), Crepis, Picris, Cichorium, and Calendula (Godineau, 1969), and in the egg of Plumbago (Cass and Karas, 1974), the mitochondria are most numerous near the filiform apparatus whereas in Quercus (Mogensen, 1972) and Agave (Tilton, 1974) they tend to be slightly more concentrated in the chalazal end of the cell. Frequently in Gossypium (Jensen, 1965a) a long crista divides a mitochondrion into two compartments, and in a large number of mitochondria a portion of the outer membrane was noted to be missing. These latter mitochondria were interpreted by Jensen as having just recently divided like those of similar appearance in the central cell.

The dictyosomes of synergids are generally numerous and have an active appearance. During development of the filiform apparatus in Gossypium (Jensen, 1965a) and Helianthus (Newcomb, 1973a), dictyosomes are most numerous in that area of the cell. In mature synergids of Capsella (Schulz and Jensen, 1968a), Crepis, Picris, Cichorium, and Calendula (Godineau, 1969), the dictyosomes are found in a micropylar-chalazal distribution whereas, in Petunia (Van Went and Linskens, 1967;
Van Went, 1970a) and in Aquelgia (Vijayaraghaven et al., 1972) they are random. Dictyosomes are most numerous in the micropylar end of Agave synergid (Tilton, 1974). The number of cisternae per dictyosome varies among species ranging from 2-8 in Agave (Tilton, 1974), 3-8 in Gossypium (Jensen, 1965a), 3-5 in Petunia (Van Went and Linskens, 1967; Van Went, 1970a), 4-5 in Helianthus (Newcomb, 1973a), 4-6 in Capsella (Schulz and Jensen, 1968a) and Aquilegia (Vijayaraghaven et al., 1972), and 5-6 in Oenothera (Jalouzot, 1975). The cisternae of Oenothera are reported to have granular contents. All synergid dictyosomes have a goodly number of vesicles associated with them except for those of Gossypium (Jensen, 1965a) which are in close proximity to the filiform apparatus. In Helianthus the large number of dictyosomes decreases somewhat after the filiform apparatus has completed development (Newcomb, 1973a).

Fertilization

Maheshwari (1950) has described the entrance of the pollen tube into the megagametophyte as occurring in one of three possible ways: 1) between the egg and one synergid, 2) between the megagametophyte wall and one synergid, or 3) directly into one synergid. Also according to Maheshwari (1950), as a rule only one synergid is destroyed by the impact of the pollen tube and the other remains intact until some time afterward, but that in some plants both are destroyed, and in others neither seems affected.

In Agave (Regen, 1941; Mogensen, 1970) and Agrostis (Muniyamma, 1976), the synergids are ephemeral and are gone before fertilization; and, in Trianthema, both the antipodals and the synergids degenerate
early so that the mature megagametophyte consists of the egg and central cell (Bhargava, 1935). The more common condition is for one synergid to degenerate and one to persist as reported for Gossypium (Gore, 1932; Jensen and Fisher, 1968), Ornithopus (Wojciechowska, 1972b), Quercus (Mogensen, 1972), several Acanthaceae (Kärilstrom, 1972, 1973, 1974a,b), and Bellis (Engell and Petersen, 1977) among many others. The persistent synergid generally degenerates following fertilization, e.g., Euphorbia (Bor and Kapil, 1975), but in some instances may not degenerate until embryo development is well-underway, e.g., Claytonia (Cook, 1903a), Vallisneria (Burr, 1903), Typha (Asplund, 1972), and Sparganium (Asplund, 1973). In a few cases both synergids are reported to be persistent, e.g., Jubaeopsis (Robertson, 1976), and in Tricyrtis (Ikeda, 1902) and Ammannia (Smith and Herr, 1971) both synergids persist until endosperm development is well-underway.

Basing his final argument concerning the function of synergids on Dahlgren's (1938) paper in which it is reported that in some plants the synergids degenerate prior to pollen tube entry into the megagametophyte and that there is a lack of synergids in Plumbago, Vogelia, and Plumbago, Maheshwari (1950) concludes that synergids are "...not essential for fertilization, and the view that they secrete substances which exercise a chemostatic influence over the pollen tube, or that they act as shock absorbers against its impact, does not rest on a sound basis."

Since Maheshwari's tome was published, however, more recent evidence has come forth and it is now conclusive that the synergids do indeed have an important role in the fertilization process.
As previously alluded to, the concept of the ovule producing some sort of chemotropic factor or mechanical contrivance involved in pollen tube growth is an old one. Most all parts of the ovule have been suggested as possibilities at one time or another, but particularly the micropyle, micropylar nucellus, and the egg apparatus itself. Welk et al. (1965) studied the chemotropic activity of several reproductive tissues of *Lilium*. For one of the bioassays, ovules were cut in half latitudinally and the two ends of the micropylar half were then assayed. The micropylar end of these halves showed greater activity than did the chalazal half.

Schacht (1857) postulated that the striations of the filiform apparatus act as mechanical guides for the pollen tube, and Ishikawa (1918) suggested that the filiform apparatus, in conjunction with substances synthesized by the synergid, act to guide the tubes chemotropically. Pluijm (1964) was the first to provide ultrastructural evidence in favor of Ishikawa's hypothesis, and since Pluijm's (1964) account of the situation in *Torenia*, several other ultrastructural studies have supported the idea that the pollen tube enters the megagametophyte via the synergids, videlicet *Gossypium* (Jensen, 1965a; Jensen and Fisher, 1967a,b, 1968; Fisher and Jensen, 1969), *Zea* (Diboll and Larson, 1966; Diboll, 1968), *Petunia* (Van Went and Linskens, 1967; Van Went 1970a,c), *Capsella* (Schulz and Jensen, 1968a), *Epidendrum* (Cocucci and Jensen, 1969b), *Quercus* (Mogensen, 1972), *Agave* (Tilton, 1974), *Aquilegia* (Fougère-Rifot, 1975), and *Bellis* (Engell and Petersen, 1977).

(Jensen and Fisher, 1968), Epidendrum (Cocucci and Jensen, 1969b), and Quercus (Mogensen, 1972), it has been shown that the pollen tube enters the megagametophyte directly at, and grows directly through, the filiform apparatus and into the synergid cytoplasm where it discharges. In none of these cases was the tube discharge reported to occur anywhere other than within synergid cytoplasm. In all cases where a pollen tube was found, the synergid that is penetrated is degenerate before or immediately after penetration, and the other synergid persists as an intact cell for a period of time that varies among species.

It is interesting to note that in Zea (Diboll, 1968), Gossypium (Jensen and Fisher, 1968), and Helianthus (Newcomb, 1973b) there is some pre-penetration change in the synergid which received the pollen tube. A unique situation is reported to occur in Oryzopsis where both synergids show some change prior to penetration and one goes on to degenerate completely. The other synergid, however, the persistent one, is the one reported to receive the pollen tube (Maze et al., 1970). The nonpenetrated synergid, except in Oryzopsis, may remain relatively unchanged following fertilization. Petunia shows no change for the first 50 hours (Van Went, 1970c), and in Helianthus no change was noted until the early heart stage of embryo development. In Torenia, however, the persistent synergid shows a size increase soon after the pollen tube enters the megagametophyte (Pluijm, 1964).

The notable cytoplasmic changes which may occur in the persistent synergid are an increase in the amount of ER in Zea (Diboll, 1968) and Gossypium (Jensen, 1968a), an increase in the number of dictyosome cisternae and associated vesicles in Epidendrum (Cocucci and Jensen,
1969b), and an increase in the number of dictyosomes in Quercus (Mogensen, 1972). The cell wall material along the common surfaces of the egg apparatus of Epidendrum disappears leaving only the two plasmalemmas separating the cells (Cocucci and Jensen, 1969b). This is in contrast to the situation in Quercus (Mogensen, 1972, 1975b; Singh and Mogensen, 1975) and Helianthus (Newcomb, 1973b) where the cell wall is completed around the entire cell following fertilization.

Haberlandt (1927) suggested that synergids secrete a substance which dissolves the end of the pollen tube, and Jensen (1965a) suggested that the chemotropic material in the synergids comes from their large vacuoles. Pritchard (1964) claims that filiform apparati also have a nutritive role for the megagametophyte by absorbing materials from the nucellus. This latter idea has received the support of Jensen (1965a), Schulz and Jensen (1968a), and Cass and Karas (1974) who base their final argument on the findings of Pate et al. (1970) that the appearance of wall ingrowths in transfer cells is temporally correlated with the availability of solutes to be transported. Maze and Lin (1975) claim that the filiform apparatus of the degenerate synergid serves to transport growth-directing compounds out of the megagametophyte and that the filiform apparatus of the persistent synergid serves to transport material into the megagametophyte. Maze and Lin (1975) also hold that the persistent synergid somehow aids in the establishment of egg polarity. Throughout history, however, most authors believe that synergids are not nurse cells and agree with the theory, most recently restated by Engell and Petersen (1977), that synergids have only one important function—videlicet to attract and receive the pollen tube.
From the early light microscope studies, it has been suggested that the two male gametes accomplish double fertilization by each one following a separate branch of the bifurcated pollen tube tip, the branches each serving as an initial guide for directing the male gametes to their respective mates (Maheshwari, 1950). Ultrastructural studies show no such bifurcation of pollen tubes. By examining an electron micrograph of a discharged pollen tube, however, it easily can be seen how such a conclusion could be drawn from light microscope observations. Figures 11 and 16 of Mogensen (1972) provide good examples.

Exact details of gamete transfer are not known. Although the fusion of gamete nuclei in plants has been described for many years (Straus-burger, 1884; Nawaschin, 1898b; Gore, 1932; Jensen, 1964), the mechanics and details of the process whereby the male gametes reach their respective mate nuclei after being discharged from the pollen tube yet remains to be elucidated. Linskens (1968), however, has postulated for sperm transfer that the plasma membrane of the sperm fuses with the plasma membrane of either the egg or central cell. By an unknown mechanism, this first fusion blocks a second fusion with the other sperm and thus prevents multiple fertilization of the first cell. This also forces the second sperm to fuse with the other cell awaiting fertilization, whichever it may be. The sperm cytoplasm is left behind in the synergid, presumably in the cytoplasm and only the sperm nucleus passes through. Cass (1973) has been able to show with present techniques that there is no apparent directional motility of sperms in Hordeum and concluded that angiosperm sperms in fact probably are not motile at all. Teleżyńska and Teleżyński (1973) believed that bursting of the pollen tube is a violent, fountainlike
reaction causing a bursting of synergid walls between synergid and egg, and between synergid and central cell. Maze and Bohm (1974) report the occurrence of an opening in the chalazal end of the degenerate synergid of *Agrostis* through which the second sperm moves into the central cell.

According to Maheshwari (1950), Strausburger (1884) discovered syngamy in plants, and from this discovery and other work, he made the following three generalizations: (1) the process of fertilization comprises the union of the male gamete with that of the egg, (2) the cytoplasm of the gametes is not concerned in the process, and (3) the sperm nucleus and the egg nucleus are true nuclei. The fate of the second male nucleus was not known until Nawaschin (1898b) and Guignard (1899) were able to demonstrate that triple fusion and syngamy are part of one process resulting in what we now know as double fertilization.

Ultrastructural data from *Petunia*, *Gossypium*, *Epidendrum*, *Quercus*, *Hordeum*, and *Helianthus* help to confirm Strausburger's second generalization in that no male cytoplasm was detected in either the zygote or central cell of any of these plants. Dryanovska (1976a,b) states that no mitochondria are contributed by sperm to zygotes of *Capsicum*, *Lycopersicon*, *Nicotiana*, and *Solanum*, but he does not mention other cytoplasmic entities. Meyer and Stubbe (1974), however, claim that they can distinguish between plastids contributed by the sperm and egg in young zygotes of *Oenothera* on the basis of their size and starch content.

In his studies on fertilization, Nawaschin (1898a,b) observed densely staining bodies either in the tip of the pollen tube or adjacent to it after the tube had discharged. Because he could determine their identity, he termed them X-bodies. Since their discovery, X-bodies have
suffered various interpretations which range from being the nuclei of the adjacent nucellar cells which are pushed into the embryo sac by the impact of the pollen tube, to the nuclei of disorganized synergids, to the conclusion of Jensen and Fisher (1968) that they represent the cytoplasm of the discharged sperm. X-bodies were first correctly identified by Satina and Blakeslee (1935) in Datura as being the nuclei of the degenerate synergid and of the vegetative pollen cell. Satina and Blakeslee’s work was first confirmed ultrastructurally by Cocucci and Fulvio (1969) in Epidendrum. Extra bodies have been reported to occur at fertilization on the surface of the egg in Oryzopsis (Maze et al., 1970) and Agrostis (Maze and Bohm, 1974) which resemble similar structures seen in the synergids of Stips tortilis (Maze et al., 1970), S. lemmonii (Maze et al., 1972), and S. elmeri (Maze and Bohm, 1973). Maze and Bohm (1974) thought that the extra bodies seen in Oryzopsis and Agrostis are the equivalent of X-bodies.

Since Cass and Jensen (1970) and Cass (1973) have shown that sperm of Hordeum are discharged as complete cells and yet no male cytoplasm has been detected in the zygote or central cell of any species examined so far except for the report of Meyer and Stubbe (1974), the sperm nuclei and the sperm cytoplasm apparently must separate from one another at some point between the pollen tube and the egg and central cell. Just how and when this occurs and what the fate of the sperm cytoplasm is, is not known.

Contact between sperm and egg is usually made before contact between the other sperm and the fusion nucleus, but triple fusion is generally completed before syngamy, and frequently the endosperm initial has
undergone one or more divisions prior to zygote cleavage (Maheshwari, 1950).

Post Fertilization Central Cell

Although the polar nuclei in Gossypium (Jensen, 1963, 1965b; Jensen and Fisher, 1967b), Zea (Diboll and Larson, 1966; Diboll, 1968), Epidendrum (Cocucci and Jensen, 1969b), Petunia (Van Went, 1970b, c), and Hordeum (Cass and Jensen, 1970) are partly fused prior to fertilization, the presence of a sperm nucleus is required for fusion to be completed. In Gossypium (Jensen and Fisher, 1967b), the sperm nucleus fuses with one of the partly fused polar nuclei and this 2n nucleus then fuses with the other partly fused polar nucleus resulting in the 3n endosperm initial. In Capsella (Schulz and Jensen, 1973), the polar nuclei may or may not be completely fused before the sperm nucleus arrives and, in Agave (Mogensen, 1970) and Helianthus (Newcomb, 1973a, b), fusion is completed prior to fertilization.

The cytoplasm of the central cell in Gossypium (Jensen, 1963), Zea (Diboll, 1968), Epidendrum (Cocucci and Jensen, 1969b), and Petunia (Van Went, 1970c) shows a marked increase in activity after fertilization. The endoplasmic reticulum and dictyosomes increase in abundance and the free ribosomes become aggregated into polysomes. In Gossypium there are plastids present in the post-fertilization cytoplasm, but few contain starch. The mitochondria are large and polymorphic (Jensen, 1963).

Shortly after fertilization in Zea, the extremely long profiles of rough endoplasmic reticulum present in the unfertilized sac are replaced by shorter, randomly distributed, variously swollen elements which become
continuous with the nuclear envelope. Plastid structure remains unaltered (Diboll, 1968). In *Epidendrum*, the plastids are elongate and the mitochondria rod shaped. The helical and ring-shaped polysomes are most abundant in the micropylar end of the cell where the endoplasmic reticulum and other organelles are absent. Single membrane-bound lipid bodies and small vacuoles are conspicuous. A large vacuole in the micropylar end of the central cell forms next to the zygote where no cell wall is present (Cocucci and Jensen, 1969b). The mitochondria of *Petunia* become large and irregularly shaped, especially near the penetrated synergid where large amounts of lipid are formed. The mitochondria now have a translucent matrix and many randomly distributed cristae and tubuli. Whereas all plastids in the unfertilized central cell contained starch, there now appear some smaller ones with no starch. A thick and irregularly shaped wall forms between the central cell and the chalazal parts of the zygote and synergids (Van Went, 1970c). In *Hordeum*, the abundant starch present in the unfertilized central cell disappears after one or two divisions of the endosperm initial (Cass and Jensen, 1970). In *Stellaria*, the wall ingrowths at the micropylar end of the cell now proliferate and more ingrowths are formed farther away from the micropyle (Newcomb and Fowke, 1973). The central cell cytoplasm around the micropylar wall ingrowths in both *Helianthus* (Newcomb and Steeves, 1971) and *Stellaria* (Newcomb and Fowke, 1973) is replaced with endosperm.

**Zygote**

At the light microscope level, the zygote appears to undergo a period of rest after which it begins to grow in preparation for the
first division. The period of rest is variable between species and is
to some extent dependent upon the environment. This period ranges from
about five hours after syngamy in *Taraxacum kok-saghys* to five months for
*Colchicum autumnale* (Furlani, 1904).

In both *Gossypium* (Jensen, 1963, 1968b) and *Capsella* (Schulz and
Jensen, 1968b), there is a decrease in vacuolar size resulting in the
zygote being smaller than the egg. The size decrease in *Capsella* is
immediate after syngamy but is, however, very limited and only temporary
whereas that of *Gossypium* results in a size decrease by one-half or more.
Not until the embryo contains approximately seventy-five cells does it
again equal the size of the original zygote (Pollock and Jensen, 1964).
In *Gossypium*, the chalazal portion of the zygote contracts where no cell
wall exists. The nucleus remains chalazal and the peripheral cytoplasm
now surrounds the nucleus in a dense mass. When the zygote is about
one-half the size of the egg, the number of plastids containing starch
and the amount of tube-containing endoplasmic reticulum increases. This
endoplasmic reticulum is continuous with the nuclear membrane but has no
association with spindle fibers (Jensen, 1963, 1965b, 1968b; Jensen and
Fisher, 1967a,b).

An increase in the amount of endoplasmic reticulum in the zygote
over that present in the egg has also been noted in *Zea* (Diboll, 1968),
*Epidendrum* (Cocucci and Jensen, 1969b), *Petunia* (Van Went, 1970c), and
*Quercus* (Mogensen, 1972; Singh and Mogensen, 1975). In *Quercus*, several
vesicles are derived from the endoplasmic reticulum cisternae. These
vesicles have a fibrillar content similar to that in the endoplasmic
reticulum cisternae. The cisternae are lamellate and are mostly near
the plasmalemma or the nucleus. The endoplasmic reticulum of the *Quercus* zygote is always associated with at least some ribosomes (Singh and Mogensen, 1975). In *Hordeum* (Norstog, 1972b), the endoplasmic reticulum is sparse in the zygote.

The number of dictyosomes and dictyosome activity increases in *Gossypium* (Jensen, 1965b, 1968b; Jensen and Fisher, 1967a,b), *Zea* (Diboll, 1968), *Capsella* (Schulz and Jensen, 1968b), *Petunia* (Van Went, 1970c), and *Quercus* (Mogensen, 1972; Singh and Mogensen, 1975). This increase in dictyosome activity is noted to be associated with cell wall synthesis in *Gossypium* (Jensen and Fisher, 1967a,b), *Capsella* (Schulz and Jensen, 1968b), and *Quercus* (Mogensen, 1972; Singh and Mogensen, 1975). Dictyosomes are conspicuously absent from the zygote of *Epidendrum* (Cocucci and Jensen, 1969b).

Following fertilization, except in *Epidendrum* (Cocucci and Jensen, 1969b), cell wall material is deposited along the common surfaces of the cells of the egg apparatus and over the chalazal end of the zygote and synergids. Although the plasmodesmata that were present between the egg and the central cell, and between the egg and the persistent synergid in *Capsella* remain in the walls of the zygote (Schulz and Jensen, 1968b), no plasmodesmata occur in the zygote walls of *Hordeum* (Norstog, 1972a) or *Quercus* (Singh and Mogensen, 1975). The micropylar wall of the zygote in *Capsella* was noted to begin producing fingerlike projections (Schulz and Jensen, 1968b).

Hordeum (Norstog, 1972a), and Quercus (Singh and Mogensen, 1975). These ribosomes are aggregated into polysomes, and in Zea (Diboll, 1968), Capsella (Schulz and Jensen, 1968b), and Epidendrum (Cocucci and Jensen, 1969b), they are ring-shaped or in the form of helices. In Gossypium (Jensen, 1967, 1968b), a second population of ribosomes appears which shows little in the way of aggregation into polysomes. The two populations of ribosomes exist simultaneously.

As a rule, the zygote is a highly polarized cell with the nucleus and organelles oriented chalazally as in Gossypium (Jensen, 1963, 1965b, 1968b), Capsella (Schulz and Jensen, 1968b), Epidendrum (Cocucci and Jensen, 1969b), Petunia (Van Went, 1970c), and Quercus (Brown and Mogensen, 1972; Mogensen, 1972; Singh and Mogensen, 1975). In Hordeum, although not highly polar, some cytoplasmic zonation is apparent (Norstog, 1972a).

The zygote nucleus is fairly large and generally has a heterogeneous chromatin content as in Petunia (Van Went, 1970c), Hordeum (Norstog, 1972b), and Quercus (Singh and Mogensen, 1975). The number of nucleoli may be one as in Capsella (Schulz and Jensen, 1968b) and Petunia (Van Went, 1970c), or two as in Gossypium (Jensen and Fisher, 1967a,b), Epidendrum (Cocucci and Jensen, 1969b), Hordeum (Norstog, 1972b), and Quercus (Singh and Mogensen, 1975). Satellite nucleoli are also present in Quercus (Singh and Mogensen, 1975). In Gossypium, where there were cases in which the two nucleoli were of unequal size, the larger of the two probably came from the egg and the smaller from the sperm (Jensen and Fisher, 1967a,b). The zygote nucleus in Quercus showed some slight lobing (Singh and Mogensen, 1975).
Mitochondria are typically numerous in the zygote. In *Gossypium* (Jensen and Fisher, 1967a,b) and *Hordeum* (Norstog, 1972a), they have a perinuclear distribution, and in *Gossypium* (Jensen and Fisher, 1967a,b) they seem to increase in number by division. Those in *Zea* appear to be swollen (Diboll, 1968) whereas in *Epidendrum* they are rod-shaped (Cocucci and Jensen, 1969b). In *Quercus* the mitochondria are endowed with well-developed cristae and distinct clear zones which presumably harbor DNA fibrils (Singh and Mogensen, 1975).

The transition from egg to zygote commonly brings about an increase in lipid content as in *Zea* (Diboll, 1968), *Capsella* (Schulz and Jensen, 1968b), *Epidendrum* (Cocucci and Jensen, 1969b), *Petunia* (Van Went, 1970c), *Hordeum* (Norstog, 1972b), and *Quercus* (Mogensen, 1972; Singh and Mogensen, 1975). Plastids and starch content may also increase as in *Gossypium* (Jensen and Fisher, 1967a,b), *Capsella* (Schulz and Jensen, 1968b), *Epidendrum* (Cocucci and Jensen, 1969b), *Petunia* (Van Went, 1970c), and *Hordeum* (Norstog, 1972b), while in *Quercus* both are rare (Mogensen, 1972; Singh and Mogensen, 1975). Some microbodies are also present in the zygote of *Quercus*.

**Aberrant Ovules**

Prompted by my discovery of two ovules from separate ovaries with a single outer integument enclosing two side-by-side inner integuments each with its own nucellus and developing megaspore, I decided to keep track of any mentioning of aberrant conditions in the papers just reviewed. This final section of the literature review, then, represents merely an accounting of these reports and does not constitute a serious attempt to
review the subject comprehensively.

By far the most commonly occurring aberrant condition is the formation of multiple megagametophytes within a given nucellus. This condition occurs so frequently in some taxa, e.g., *Citrus* or *Theobroma*, that it may be considered as normal by these groups. In most instances, however, multiple (usually two) megagametophytes are sporadic enough in their occurrence to be considered aberrant, e.g., *Delphinium* (Mottier, 1895), *Gossypium* (Gore, 1932), some legumes (Martin and Watt, 1934), *Lilium* (Fourcroy, 1949), *Ornithogalum* (Czapik, 1972), *Malcomia* (Prasad, 1975), and *Persea* (Sedgley, 1976). The occurrence of three mature megagametophytes in a given nucellus is indeed rare, having been reported for one count in *Paeonia* (Cave et al., 1961) and two counts in *Ulmus* (Lester and Lee, 1974). *Paeonia* and *Ulmus* also produced two-megagametophyte ovules, and in both taxa both megagametophytes may be fertilized.

Multiple fertilizations of one ovule are also reported for *Persea*, and Sedgley (1976) postulates that the number of pollen tubes reaching the ovary is closely related to the number of megagametophytes in the ovule, there being only one ovule per ovary. While only one pollen tube is able to reach the ovary in normal, single-megagametophyte, single-ovule individuals, two pollen tubes were found in the micropyle of 18 percent of the two-megagametophyte, single-ovule individuals. According to Sedgley (1976), of the approximately 66 pollen grains that germinate on the style, half form swollen tips and go no further than the stigma base, and the remaining tubes go no further than half to three-fourths of the way down the style so that only 1-2 reach the ovary, depending in part
on the number of megagametophytes present. Fulvio and Cave (1964) report seeing two pollen tubes reach the megagametophyte of *Blandfordia* also.

Aposporic megagametophytes have been reported for *Ornithogalum gussonei* by Żabińska (1972), and Czapik (1972) reported the fusion of two *O. umbellatum* ovules but gave no particulars. Twin nucelli have been noted to occur in *Moringa* (Puri, 1934), *Ornithopus* (Wojciechowska, 1972a), *Ornithogalum nutans* and *O. nanum* (Karagozova and Van Khankh, 1972), and *Persea* (Tomer and Gottreich, 1976). Tomer and Gottreich also report several other unusual conditions for *Persea*, i.e., egg apparatus without a filiform apparatus, additional nuclei--from few to 20-- within the central cell, and the occurrence of two egg apparatus within one ovule. Several cases of four-celled egg apparatus have been reported for *Morninga* (Puri, 1934), and one aberrant megagametophyte of *Acer* had an egg apparatus consisting of three eggs and two synergids (Haskell and Postlethwait, 1971). Cooper (1943) demonstrated in several species of *Lilium* and in one of *Nicotiana* that a synergid was stimulated to divide resulting in haploid-diploid twins. The haploid individual usually deteriorates at an early stage, but some did develop more fully. Wojciechowska (1972a) found some *Ornithopus* ovules without a megagametophyte as did Mogensen (1975a,b) in *Quercus*. Mogensen (1975a) found four types of abortive ovules in *Quercus*. Simons (1974) claims that ovule abortion in apple is the result of poorly developed placentae which are unable to support functional ovules. Lloyd (1899) stated that it is common to find completely developed megagametophytes in the normal position plus in the chalaza and even in the funicle of *Asperula*. Finally, the transformation of filament to ovule
tissue has been reported to occur in *Nicotiana* (Bhat and Krishnamurthy, 1956; Krishnamurthy and Rao, 1960) and *Solanum* (Sirohi et al., 1964; Pullo and Slusarkiewicz, 1975).
MATERIALS AND METHODS

Plants of *Ornithogalum caudatum* Ait. were grown in the Department of Botany and Plant Pathology greenhouse at Iowa State University, Ames. Tissues from gynoecia ranging in age from buds of the third floral plastochron up through mature seeds were studied in varying degrees of detail. Samples were collected as needed between November, 1974 and June, 1977.

**Light Microscopy (LM)**

All material included within this study was examined in detail with light microscopy.

**Paraffin processing**

To become acquainted with *Ornithogalum*, whole buds (for younger stages) and whole gynoecia (for older stages) were fixed in FAA and subsequently processed by standard paraffin methods (Sass, 1958). Paraffin sections were cut at a thickness of 10 μm on a rotary microtome and then stained with safranin and fast green (Sass, 1958). As this material was strictly for orientation purposes, no micrographs were taken of paraffin embedded specimens.

**Resin processing**

Most of the material examined with light microscopy was embedded in resin, and all light micrographs are of resin processed material. Whole buds (for younger stages) and whole flowers (for older stages) were cut

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1 See Appendix C for formulae and schedules for buffers, fixatives, embedments, and stains.
off the parent plant at the pedicular node with a razor blade. The buds and flowers were then further dissected on discs of blotter paper saturated with 1% glutaraldehyde. Dissected material was immediately placed into glutaraldehyde buffered with sodium cacodylate. Following fixation, tissue was rinsed in buffer, dehydrated in ETOH, and embedded in Spurr's resin (Spurr, 1969).

Blocks for light microscopy were sectioned with dry glass knives on a Reichert Om-U2 ultramicrotome at a thickness of 2 μm. Sections were plucked from the knife in serial order with tweezers and placed in three rows of five sections each on dry glass slides. Sections were viewed in this state with phase optics to select appropriate sections for staining. Each section selected by this method was then individually transferred to a second glass slide with tweezers, one section per slide. Serial order was thus maintained. Each section was next floated on a drop of glass-distilled water and heated over a spirit lamp to expand it. After the water evaporated, the slide was heated again over a spirit lamp to adhere the section to the slide.

Resin embedded sections were stained with a variety of dyes including acid fuchsln (Feder and O'Brien, 1968), aniline blue black (Fisher, 1968), azure B (Flax and Himes, 1952), basic fuchsln (Humphrey and Pittman, 1974), methylene blue-azure II (Humphrey and Pittman, 1974), naphthol black (Author, Appendix C), Paragon (Spurlock et al., 1966), and toluidine blue O (Trump et al., 1961). Following staining, slides were rinsed, dried, and immersed overnight in xylene. Coverslips were mounted with Ficcolyte.
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1. See Appendix C for formulae and schedules for buffers, fixatives, embedments, and stains.
Transmission electron microscopy (TEM)

Specimens for TEM were processed in the same manner as those for LM up through the buffer rinse following glutaraldehyde fixation. At that point, tissue for TEM processing was post-fixed in OsO$_4$, rinsed in buffer, stained <<en bloc>> in aqueous uranyl acetate, dehydrated in ETOH, and then embedded in Luft's epoxy resin (Luft, 1961) via propylene oxide. Silver to silver-gold sections were cut with a DuPont diamond knife on a Reichert Om-U2 ultramicrotome. Sections were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and then examined with a Hitachi HU-11C electron microscope.
OBSERVATIONS

Carpels

Initiation and morphology

Each carpel is initiated as an individual U-shaped primordium by the floral meristem (Fig. 1). In addition to the floral meristem, there are meristematic zones which form along the carpel margins. These meristems contribute cells to the carpels proper and to the ovules. In tricarpellate flowers, the carpels are arranged in a triangle with their margins facing the center of the triangle (Fig. 1). In bicarpellate flowers, however, the margins of one carpel oppose those of the other carpel and an elliptical ovary results.

The tricarpellate condition is predominant, occurring in about seventy percent of all flowers observed. Both bicarpellate and tricarpellate flowers may occur in the same inflorescence. In most instances, the first-formed flowers tend toward being tricarpellate while the later-formed flowers, and especially those formed as the inflorescence becomes spent, are more frequently bicarpellate.

In addition to a variance in the number of carpels, other floral parts may differ in number between individual flowers as well. Stamens vary from the normal six to a low of four and a high of eight. Tepals show the same range of variance. Quite commonly, the last-formed flowers are deformed, the floral parts being misshapen or only partially formed. In several of these flowers, the gynoecium was found to be monocarpellate, and, in one such flower, the carpel was growing up around a lobe of one of the anthers (Fig. 2).
Fusion and ovule initiation

The process of carpel fusion is not continuous once it begins, but rather it is initiated at two loci at two different points in time. The process begins very soon after the primordia are initiated. The first contact occurs behind the leading edge of the margin along the base of the abaxial surface where adjacent carpels are in their closest proximity (Fig. 3). Fusion here (henceforth referred to as fusion zone 1) occurs between only about 5-6 cells from each primordium in the radial plane (Figs. 4, 5), while in the axial plane fusion will eventually extend all the way up to the tip of the style. Although the carpel bases fuse at an early stage, the tips, which later produce the style and stigma, remain free until well after the ovules are initiated and the style has begun to elongate (Figs. 6, 7).

Following fusion in zone 1, the carpel margins continue to grow toward the center of the ovary and at the same time four meristematic zones become evident: two at and two interior to the two margins of each carpel (Fig. 8). The two larger zones are located on the adaxial side of the carpel slightly behind the leading edge of each margin (Figs. 9, 10, 11). Activity by these meristems (the placenta) produces the ovule primordia. Placentation is thus axile. The primordia are initiated in an acropetal sequence (Fig. 6) and are first discernible as small swellings on the adaxial surface of the carpel (Fig. 3). Primordia are initiated prior to the second phase of carpel fusion.

Continued activity by the smaller two meristematic zones, located at the leading edges of the margins (Fig. 8), results in the tips of the margins meeting in the center of the ovary (Fig. 12). After the
tips touch, they fuse (fusion zone 2) and form the large central core of tissue (Figs. 13, 14, 15) which traverses the entire length of the ovary axis. This central core terminates at the ovary-style boundary. The radial segments of the now-fused carpels form the septa between locules. The septa, like the central core, extend from the base of the ovary up to the ovary-style boundary. *O. caudatum* is thus eusyncarpous.

**Septal nectary**

An additional feature of the carpels in *O. caudatum* is that fusion between adjacent carpels is not complete in the radial plane. Adjacent carpels remain free from one another for a short span in the region of the septum between the two fusion zones. The result is a narrow chamber that extends one-third to one-half the radial distance of the septum (Figs. 13, 14, 15) and the entire axial distance (Figs. 6, 16, 17).

The cells which line the chamber retain their appearance as epidermal cells (Fig. 18). Cells which fuse, however, lose their former identity as epidermal cells and, except for position, are almost indistinguishable from other cells of the cortex (Fig. 5). Careful scrutiny of the fusion zones, however, will reveal the suture (Fig. 14).

Another character of interest in respect to these septal chambers is that the cells which line them are secretory. The cells fill with cytoplasm and subsequently secrete a substance into the chamber lumen. This material stains positively for protein with both aniline blue black and napthol black (Fig. 19).

The carpels become morphologically (but not anatomically) mature after they form a grooved ridge to the exterior of the suture zone
(Figs. 4, 14). After this ridge is formed, there are several major anatomical developments, to be presented later, that are yet to occur before the carpels dehisce.

Epidermis

The carpels are surrounded by a uniseriate epidermis. When the carpels are seen in cross-section, the epidermal cells appear as rather elongate, columnar cells (Figs. 20, 21). Wall fibrils occurring at the junction of the radial and the outer tangential walls have an undulatory appearance (Figs. 22, 23). The outer tangential wall is two to three times thicker than the other walls (Figs. 20, 21) and is covered externally by a well-defined cuticle (Figs. 24, 25).

Cuticle The cuticle is composed of three distinct layers—inner, middle, and outer. Before the cuticle attains its mature proportions, the inner layer is approximately the same thickness as the middle layer (Fig. 26). The immature inner layer, however, is easily discerned from the middle layer and the cell wall by its relatively greater electron transparency and its somewhat scalloped appearance (Fig. 26). With age, the inner layer increases both in electron density and in width (Fig. 25) but does not lose its scalloped appearance (Fig. 27). When mature, this layer comprises the greatest proportion of the cuticle and is seven to eight times thicker than the middle layer (Figs. 25, 27).

The middle layer, termed the outer cuticle proper, appears to be fairly homogeneous (Fig. 28) and is composed of 2-3 lamellae (Figs. 26, 27). The outermost layer, termed the epicuticular wax layer, appears in cross-section as a thin sheet of material (Figs. 28, 29). This layer
first becomes undulatory (Figs. 24, 27) and then folded and convoluted (Figs. 28, 29).

**Stomates** Prior to and for a short time after anthesis, the carpels are devoid of any stomates. After the flowers have been open for a short while, however, partially sunken stomates develop (Figs. 20, 21). In that no subsidiary cells form in conjunction with the guard cells, the stomates are of the anomocytic variety.

**Lipotubuloids** Epidermal cells, although highly vacuolate, contain aggregations of osmiophilic bodies (Figs. 20, 21). TEM micrographs show the bodies to be single spherosomes (Fig. 30) or clusters of spherosomes (Figs. 31, 32, 33). The spherosomes are bound by a single, half-unit membrane (Fig. 31), and some of them contain small, very densely staining granules (Figs. 31, 32). Some of the spherosome aggregations appear within the cytoplasm proper or are cytoplasmic extensions into the vacuole (Figs. 32, 32). The cytoplasm associated with the aggregated spherosomes contain RER, plastids, microtubules, ribosomes, and dictyosomes (Figs. 32-47).

As carpels age, the spherosomes appear to be catabolized. Individual spherosome digestion occurs in a centrifugal manner from one (Figs. 32, 33, 37, 40) or more (Fig. 44) loci. Digestion within the aggregation as a whole, however, seems to proceed centrifugally with the centermost spherosomes being completely digested first (Figs. 21, 44). In the oldest carpels observed with such epidermal inclusions (these carpels contained seeds with mature embryos, but had not yet dehisced), the spherosomes were approximately half digested and were arranged in a ring (Fig. 44). Digested regions within some of the
spherosomes were traversed by thin, fibril-like structures (Fig. 41).

Tubules that fall within the size range of microtubules (22-24 nm) were found associated with the spherosomes. These tubules seem to interconnect closely proximate spherosomes (Figs. 34-36, 40), and in several instances tubules were seen to remain intact between an undigested spherosome and one that had been completely digested (Figs. 32, 37, 40). The tubules do not seem to be arranged in any particular fashion, but those between spherosomes are often parallel with one another (Fig. 40). However, tubules may be found lying at right or oblique angles to each other as well (Figs. 41-47). Dehisced carpels are devoid of spherosomes.

Cortex

Vascular tissue Carpel vasculature consists of five major veins, divided into three principal sets, and a network of minor veins. The largest and most prominent vein is the single dorsal vein located in the center of the carpel (Figs. 48, 49). The two ventral veins are the next largest (Figs. 48, 51, 52), and one each is located at the tip of a margin. The smallest of the primary veins are the two lateral veins. They are found at the juncture where the carpels fold inward along fusion zone 1 (Figs. 48, 50).

Because of its large size, the dorsal vein causes a bulge to form on the adaxial side of the carpel between ovules (Figs. 48, 49). The procambial strand from which this vein differentiates is the first to form, and it also contains the first recognizable xylem and phloem elements (Fig. 48). The bundle is bicollateral and develops in the usual fashion with phloem on the abaxial side and xylem on the adaxial side.
Phloem differentiation is centripetal and xylem differentiation is centrifugal. Ventral bundles are more complex than the dorsal vein in regard to xylem and phloem distribution within the bundles. Vascular bundles which feed the ovules branch off into funiculi from the ventrals. The two lateral veins are the smallest and least complex of the major veins. They are evident as procambial strands by the onset of megaspore meiosis, but do not develop further into vascular tissue until the megagametophyte is mature. At this time, however, both xylem and phloem differentiate in the normal manner.

Although the procambial strands which will eventually produce the minor veination are evident at the time of meiosis, development beyond the procambial stage is delayed until well after anthesis. The minor veins run at right or oblique angles between the dorsal vein and the lateral veins, but do not connect with the ventrals.

Ultrastructurally, the procambial cells which are the precursors of xylem cells show numerous mitochondria, ribosomes (both as single ribosomes and as polysomes), and dictyosomes. The dictyosomes commonly appear cupped around small whorls of ER and small clusters of ribosomes. There are numerous vesicles associated with the dictyosomes, most of which occur around the curved side, i.e., away from the side encircling the ER and ribosomes.

In several instances, young tracheary elements were noted to contain material which stained positively for protein with naphthol black. When seen with the TEM, this material appears as a generally homogeneous, finely granular substance. There are, however, a few
regions of greater electron transparency interspersed within the dense matrix (Fig. 56).

Differentiating sieve tube elements are identified ultrastructurally by their sieve plates (Figs. 60, 62), p-protein (Figs. 60, 63), and the crystalloids characteristic of sieve tube plastids (Figs. 60, 61, 64, 65). The crystalloids are probably carotenoids of some type in paracrystalline form. Crystalloids may demonstrate either unidirectional (Figs. 64, 65) or bidirectional (Fig. 61) periodicity depending on the plane of section. The plastids which contain the crystalloids also contain some small spherical structures 40-45 nm in diameter. The spheres may occur singly, in small clusters, or in short chains (Figs. 64, 65). They appear to form by pinching off from the inner of the two membranes surrounding the plastid (Fig. 64).

Associated with all vascular tissue in the carpels following anthesis, and especially with the minor veins, are very unique and rather curious cells. When first formed, they have exceptionally dense and highly osmiophilic cytoplasm (Figs. 66, 68). With age, the cytoplasm of these cells becomes thinner, and the cells become progressively more vacuolate (Figs. 67, 69) until the center of the cell is occupied by a large vacuole. During each cell's transition to the vacuolate stage, the tonoplast forms a complex series of loops and folds into the central vacuolar region (Figs. 70-73). In some regions, the loops open out around small islands of cytoplasm (Fig. 73). The islands have a variety of contents ranging from mitochondria (Fig. 70) or RER (Fig. 72) to polysomes and other granular or fibrillar contents (Fig. 73). In one instance, an intricate whorl and folding of SER is included within one
of these loops (Fig. 71).

These special cells are packed with abundant mitochondria, all of which have a very dense stroma and numerous cristae. Degeneration of the mitochondria as the cells enter the vacuolate stage results in the formation of numerous small vacuoles in the peripheral cytoplasm (Fig. 69). Degeneration seems to occur with the stromal matrix disappearing first. This is followed by a progressive loss of cristae caused by degeneration of the inner mitochondrial membrane. In most instances degeneration seems to be polar, beginning at one side of a mitochondrion and then progressing across to the opposite side. In a few instances, however, degeneration appears to be bipolar (Fig. 69).

These peculiar cells also contain plastids, and, although they are few in number, the plastids have a very dense ground substance. Some plastids may have several osmiophilic inclusions (Fig. 69). In addition to mitochondria and plastids, free ribosomes, polysomes (Figs. 74-81), and whorls of SER (Figs. 78, 81) are found. These whorls of SER are seen near the cell walls, and, although smaller, they look very similar to the SER complex included within one of the tonoplast loops as described earlier (Fig. 71). The nuclei are highly lobed (Fig. 77).

The ER of companion cells, though not abundant, is mainly of the smooth type (Figs. 82, 83), and an occasional dictyosome may also be seen (Fig. 82). Plasmodesmata between these cells are sometimes rather common (Fig. 68), and in one instance a branched and rather complex plasmodesma was found (Fig. 84).

**Plastids** Prior to anthesis, amyloplasts may be found in a few cortical cells, but this is not common and each amyloplast that is
present usually contains only one starch grain (Fig. 85). Coincident with the post-anthesis formation of stomates, chloroplasts develop within cortical cells of the carpels (Fig. 86). The cells often become so packed with chloroplasts that the chloroplasts press against one another (Figs. 87, 88) and may even partially conform to the shape of their neighbors (Figs. 87, 88).

The chloroplasts develop from proplastids via a progressive proliferation of the membranes which will eventually make up the thylakoid system (Figs. 89, 90). Vesicles and swollen membrane cisternae are often found in young chloroplasts (Fig. 90) as well as in older ones (Figs. 91, 92). The invaginations of the inner membrane are usually located between the thylakoid membranes and the inner membrane of the chloroplast envelope (Figs. 90-92). They appear to be either fusing with, or pinching off from, the inner membrane (Figs. 90, 91).

Chloroplasts also contain lipid droplets (Fig. 93). The number of droplets seen per section varies greatly between chloroplasts, ranging from zero (Fig. 87) to more than twenty. In some of the chloroplasts from older carpels enclosing nearly mature seeds, the droplets appear to become digested (Figs. 94-96), and some may display fine membranes and fibrillar structures.

ER (Fig. 97) and microbodies (Figs. 87, 96, 98) are occasionally seen in close association with the chloroplasts. In addition, vesicles of either ER (Fig. 97) or dictyosomal (Fig. 98) origin are commonly found near the chloroplasts as well (Figs. 91, 100).

**Crystalliferous idioblasts** Carpeels contain two types of idioblastic cells (Fig. 101). One type is crystalliferous and produces
calcium oxalate raphide crystals (Fig. 103). Crystal cells are found
quite commonly in groups of two (Fig. 102) or three (Fig. 101) cells,
and are usually located in the peripheral region of the carpels. Crystals
may be oriented parallel with (Fig. 104) or perpendicular to (Fig. 101)
the ovary axis. In young carpels (preanthesis), crystal cells are easily
distinguished from other cortical cells by their much greater size (Fig.
101). In older carpels, however, neighboring parenchyma cells may attain
as great a size as crystalliferous ones (Fig. 104). In older carpels,
therefore, one must examine several serial sections to determine whether
or not a given cell contains crystals.

Crystals usually are in well-organized bundles (Fig. 103) with the
crystals occurring in rows (Figs. 102, 105, 106). But in some instances,
bundles are rather loosely packed and the crystals are in a more random
arrangement (Figs. 104, 107). Crystal bundles develop in the middle of
the large central cell vacuole within a matrix of material which stains
positively for both protein, RNA, and carbohydrate (Figs. 102, 105).
Although the crystal bundles never fill the vacuole and individual crys­
tals are never in contact with the tonoplast, the matrix often does
touch it (Figs. 102, 105).

Ultrastructurally, the matrix has a fibrillar and somewhat flocculent
appearance (Figs. 108, 109, 114, 115). The matrix occurs around (Fig.
107) and between (Figs. 107, 109) individual crystal chambers. High
magnification reveals the presence of minute 54 Å spheres and tubules
in the flocculent substance (Figs. 108-112, 114, 115). In older carpels
(post fertilization with young seeds), the matrix changes in character
somewhat. It takes on a more homogeneous appearance by becoming more
finely fibrillar with only small, scattered patches of flocculence (Figs. 106, 126-128). Spheres and tubules, like those seen in younger stages, are still recognizable as such in the patches (Figs. 126-128). The patches now occur only in that part of the matrix which surrounds the bundle and not between crystals (Figs. 106, 126-128).

Crystals are developed within their own individual chambers which are formed by a series of lamellae (Figs. 106, 107). The number of lamellae per chamber is highly variable, differing not only between separate chambers (Fig. 106) but between different radii of a given chamber as well (Figs. 108, 109, 115-117, 132, 134). For any one radius, the lowest number seen was two (Fig. 122), and the highest number was thirteen (Fig. 108). When viewed in both cross section (Figs. 108, 113) and longitudinal section (Figs. 116, 117), it is evident that the lamellae are platelike and thus superficially resemble successive periderms rather than being sheetlike and completely ensheathing the chamber.

The innermost lamella is different from the others. It appears as a thinner, more densely staining line than the others (Fig. 131), but it does not have a substructure similar to the others. A further difference between the innermost lamella and the others is that the innermost one seems to be a continuous layer that lines the entire chamber (Fig. 131).

Lamellae, except for the innermost one, may begin and/or terminate abruptly (Figs. 108, 109, 113, 115), or they may anastomose with other lamellae and then terminate (or begin) (Figs. 116, 117, 132, 134). The abrupt endings, the fusions, and, in some cases, the overlapping of the lamellae from one side of the chamber by those of another side (Figs.
107, 108, 114) result in some very interesting lamellar patterns (Figs. 107-125, 131-134).

High magnification reveals that the lamellae are composed of chains of small spheres which are 61 Å in diameter (Figs. 108, 110, 113, 122, 123). Complementing these intralamellar spheres are interlamellar tubules that appear circular in cross-section (Fig. 108). The tubules, 54 Å in diameter, are regularly spaced with a center-to-center distance of 108 Å. Although not discernible between all lamellae, tubules can be seen in most instances. A developmental study of the crystals was not conducted.

**Idioblasts—A new type** The other type of idioblastic cell found in the carpels is a heretofore undescribed cell type. These cells are not unique to carpels, however, as they occur in the style, anthers, and tepals. Only a few observations of these idioblasts were made in tissues other than carpels, so the following descriptions are restricted to idioblasts from the carpels.

The idioblasts differentiate acropetally from parenchyma cells near the base of the ovary. The first cells to differentiate at any given level along the ovarian axis form next to, or very close to, the lateral veins (Figs. 135-137). Concurrent with acropetal differentiation is a radial differentiation with cells differentiating out from the lateral veins in all directions (Figs. 140-142). Idioblasts eventually are found throughout the carpels (Fig. 141) but are most dense in that region around the lateral veins, septal nectaries, and suture zones (Figs. 143, 144).

The first indication of a cell differentiating into one of these idioblasts is a progressive increase in cytoplasmic granularity in the
center of the cell (Figs. 145-147). Accompanying the increase in granularity is the formation of a very densely staining spherical structure (Fig. 146). This structure seems to be the center around which further changes take place in the cytoplasm that make these cells unique.

Mature idioblasts are found in two basic patterns (Figs. 148-152). In one pattern there is a dense mass, generally in or near the center of the cell with arms radiating out from it (Figs 153, 154). The arms may either taper distally (Fig. 154) or they may terminate in a knob (Fig. 153). The knobs in turn may have secondary arms that connect with the secondary arms from other knobs to form a ring very near the perimeter of the cell (Fig. 153). The second pattern consists of a ring of dense material which forms a circle concentric with the cell wall. This ring is usually midway between the center of the cell and the cell wall (Fig. 150), and, in some instances, there may be two rings end to end (Fig. 152). The rings in both types of patterns usually have slender tapering secondary arms that radiate centrifugally (Fig. 150).

The dense masses in the center of the first pattern, the dense rings in the second pattern, and the secondary arms in both patterns stain intensely for carbohydrate with basic fuchsin (Figs. 153, 154). Less dense and somewhat diffuse-appearing material is often seen around (or between) the rings and the arms. This material stains positively for protein with both aniline blue black (Fig. 153) and naphthol black (Fig. 154). With age, the arms and other cell contents continually become more diffuse in appearance (Figs. 149, 154) until they disappear.

Modifications for transport Following anthesis, transfer-type wall ingrowths, or Wandlabrinthes, occasionally form in a few cells of
the cortex (Fig. 165). In most instances, however, such changes in wall anatomy are limited in size with the walls becoming slightly undulatory or rugose (Fig. 164).

Paramural bodies are found in some cells (Figs. 164, 166) and may be associated with primary pit fields which contain many plasmodesmata (Figs. 155-162). The vesicles and/or tubules within the paramural bodies are defined by membranes that have small spherical structures within them. Vesicles limited by membranes with a similar substructure are found in the cytoplasm of the cells on either side of the wall (Fig. 164) or within the wall itself (Fig. 166). In one of the paramural bodies, the plasmalemma has invaginated into the central vacuole forming a large spherical structure with a short stem. Within the spherical portion are numerous concentric whorls of membranes with swollen cisternae. There are also some spherical structures which may be tubules seen in cross-section (Figs. 163, 166).

The nuclei of most cortical cells do not have crystalline inclusions (Figs. 167-172), but some do (Fig. 173).

**Endothelium and dehiscence**

The adaxial epidermis of carpels forms the endothelial layer which lines the locules (Figs. 174-176). The cells are cuboidal to rectangular (Figs. 175, 177) and divide anticlinally as the locules enlarge (Fig. 177). When ovules are fully anatropous the inner integument of some presses against the endothelium (Figs. 174, 175, 178) so that their micropyle opens directly onto it (Fig. 179).
As seeds approach maturity, carpels undergo some changes which allow them to dehisce. One of the first things to occur is a general degeneration and collapse of epidermal and cortical cells (Figs. 180, 181). The outer tangential wall of the endothelium is greatly thickened and has a heavy cuticle (Fig. 182). As carpels dry down further, both this wall and its cuticle continue to increase in thickness (Figs. 183, 184), and the cuticle extends along the radial walls between adjacent cells (Fig. 186). An interesting point to note is that endothelial cells are the last cells to degenerate (Figs. 180-185). In the final, dehisced stage when carpels are dry, endothelial cells are devoid of cytoplasm, the outer tangential wall has curled inward in the shape of a "U", and the cuticle has reached its maximum thickness (Fig. 185). Staining with azure B (Fig. 185) and also with methylene blue-azure II and basic fuchsin reveals the presence of several layers in the walls (Fig. 185) that were not as distinctly evident in previous stages (Figs. 182-184).

In dry carpels most of the cells are completely devoid of cytoplasm, but some cells do have cytoplasmic remnants. The remnants appear either as a homogeneous, finely granular material (Figs. 187, 188) or as somewhat spherical bodies with a finely fibrillar matrix which is more dense in the center than around the periphery (Figs. 187, 188). In some cases it appears as though the less dense peripheral area has been digested leaving behind a vacuolelike area around the dense core (Figs. 187, 189). These bodies appear to be (Fig. 189) or to have been (Figs. 187, 189) bound by a membrane.
Obturator

A proliferation of tissue at the base of the funiculus and at the tip of the carpel margin results in formation of a prominent obturator. The initial indication of obturator development occurs coincidentally with prophase of megaspore meiosis (Figs. 190, 191).

First, endothelial cells at the base of the funiculus divide antipodally and then subsequently elongate slightly to form a small swelling (Figs. 190, 191). Continued development in this region produces that part of the obturator which lies at the opening of the micropyle (Figs. 192-197). The other portion of the obturator is derived from growth of the tips of the carpel margins between the two rows of ovules (Figs. 192, 193, 197, 199). This portion of the obturator appears to be one to two cell layers deep (Figs. 197, 199), whereas that portion at the base of the micropyle is generally uniseriate (Figs. 196, 197) but also may be multiseriate (Fig. 195).

When fully mature, the obturator forms a pad of tissue that extends vertically from the base of the ovary to slightly above the apicalmost ovule (Figs. 198, 200). Horizontally, the obturator extends all the way across the two rows of ovules (Figs. 197, 199, 201, 202).

Cells forming the surface layer of the obturator are columnar (Figs. 196, 197, 199, 203). Cytoplasm in these cells is usually dense (Figs. 203-205) and contains ER, dictyosomes, and numerous small vesicles as well as other organelles (Fig. 206). A large vacuole is commonly found in the distal end of the cell (Figs. 203, 208). The cells are secretory and produce a substance which coats their surface (Figs. 202-210). The material first appears as small globules giving the cells a
warty appearance (Fig. 208). At cell maturity, however, the obturator is covered with a thick layer of the substance and no longer appears warty (Fig. 203, 204, 210).

When pollen tubes reach the ovary after growing down through the style, they grow along the surface of the obturator (Figs. 204, 205, 209, 210).

**Style and Stigma**

**Epidermis**

The stylar epidermis is a uniseriate layer of columnar cells (Fig. 211). The outer wall is very broad and is covered by a thick cuticle (Figs. 211-217). The cuticle is similar to that described previously for the carpels. When young, the outer wall is relatively smooth but, with age, the external side becomes undulate (Figs. 213-215, 217), and at maturity it is rugose (Fig. 218). Folds seem to occur first in the clefts found to the exterior of the junction between two cells (Figs. 212, 216). To the inside, epidermal cell walls form transfer-type ingrowths along all surfaces (Figs. 218, 221, 222). Plasmodesmata are found in groups and may be ramified forming complex networks (Figs. 219, 221).

Cytoplasm in stylar epidermal cells is found in a narrow but dense layer around the central vacuole (Fig. 218). Numerous thin arms of cytoplasm project into, or completely across, the vacuole (Figs. 218-222). Cytoplasm in these arms contains ER, plastids, dictyosomes, vesicles, and ribosomes (Figs. 220-222). Abundant ER, primarily RER, is found in the peripheral cytoplasm as are plastids, mitochondria, and
ribosomes, both as monosomes and as polysomes (Figs. 220-222). Lipid bodies are seen in plastids (Fig. 222) and are found in the cytoplasm as spherosomes. Spherosomes may often be quite numerous in epidermal cells (Fig. 211).

Cortex

The stylar cortex is composed of three to six layers of parenchyma cells. Vascular tissue which feeds the style is found within the cortex. It consists of one collateral bundle in each lobe of the style (Fig. 223). Each bundle is a continuation of the dorsal vein from the subtending carpel.

In longisection, cells of the cortex often appear to have two large vacuoles. A band of cytoplasm containing the nucleus, at or near the cell center, appears to separate the two vacuoles (Figs. 224, 225). This appearance is probably a result of the plane of section, however, as cross-sections show only one vacuole with the nucleus projecting into it (Fig. 226). In many cells, the tonoplast bulges into the vacuole forming large secondary vacuoles (Figs. 224, 225, 227). ER may be seen in the basal portion of some of these vacuoles (Fig. 227). Both the primary (Figs. 226, 227) and the secondary (Figs. 224, 225, 227) vacuoles contain a substance that is fibrillar to flocculent in its appearance. This material stains positively for protein, lipid, and RNA with a variety of dyes (Figs. 228, 229).

Within the cytoplasm are numerous small vesicles (Figs. 226, 227) and numerous spherosomes (Figs. 223, 230). Many spherosomes project into the central vacuole and are quite noticeably less dense around their
vacuolar side (Fig. 230). Spherosomes which do not project into the vacuole have a homogeneous appearance (Figs. 227, 230).

Microbodies and amyloplasts (Fig. 231) are conspicuously rare or absent (Figs. 224, 225, 230). And, whereas dictyosomes and ER are sparse, ribosomes are abundant and occur as both monosomes and polysomes (Fig. 227). Nuclei are commonly multinucleolate (Figs. 224, 226) and many have crystalline inclusions (Figs. 232-235). Plasmodesmata between cortical cells are not common, but in one instance there were three closely spaced clusters of ten, thirteen, and seventeen plasmodesmata (Fig. 236).

Endothelium and stylar canal

Early stage The epithelial layer which lines the stylar canal is secretory and eventually fills the lumen with its secretory product (Fig. 237). When the style is first formed, opposing sides of the endothelium in each arm of the canal closely approximate one another but do not touch (Fig. 238). Prior to its secretory phase, the endothelium is covered by a cuticle (Fig. 239), but as the cells become secretory the cuticle is sloughed (Figs. 240-243). Cells toward the center of the canal become secretory first, thus sloughing of the cuticle proceeds centrifugally (Figs. 240-243).

Endothelial cells in presecretory (Fig. 239) and early secretory (Figs. 244-246) phases have a large central vacuole and a narrow peripheral band of cytoplasm. Before cells enter their secretory phase, they commonly contain numerous lipid bodies (Figs. 240, 241), some of which may be quite large (Figs. 244, 245). Actively secreting cells, however, may have only a few or no lipid bodies (Figs. 242, 243, 247-250). Similar
to the situation in cortical cells, spherosomes which project into the vacuole appear to become digested on their vacuolar side first (Fig. 239). Other spherosomes, those that do not project into the vacuole, seem to become digested evenly in centripetal fashion (Figs. 251, 252). In very young secretory cells the tonoplast frequently proliferates and forms secondary vacuoles (Fig. 246).

The first organelles to become abundant in young endothelial cells are ribosomes and ER, most of which is RER (Fig. 253). Ribosomes are present both as monosomes and polysomes (Figs. 251, 253) and some of the ER is in direct contact with the plasmalemma (Figs. 251, 252). Microbodies are present also but not in large numbers (Fig. 253).

The secretory product in the canal at this time stains positively for lipid with osmium, protein with naphthol black (Figs. 244, 245), and ultrastructurally it appears as a network of fibrils (Fig. 254). In addition to this reticulate material, there are numerous osmiophilic spheres in the canal (Figs. 246, 252) as well as remnants of the cuticle (Figs. 253, 254). The dense spheres also are found within a translucent zone along the outer portion of the wall of the secretory face (Fig. 251), but they are not found within the endothelial cells proper.

**Filling stage** As endothelial cells fill with cytoplasm, their central vacuole becomes reduced and is divided into several smaller ones (Figs. 247, 255). Mitochondria (Fig. 255) and plastids (Fig. 256) become numerous during this phase of development (still preanthesis), and ER changes from rough to smooth (Fig. 255). Plastid stroma becomes very dense, the inner envelope membrane forms numerous cisternae, and the plastids fill with starch (Figs. 258-261). In addition to starch grains,
plastids contain numerous lipid bodies (Figs. 260, 261) and usually are surrounded by SER (Figs. 258-261). Mitochondria (Fig. 257) do not become ER ensheathed. Microbodies are present but are not too abundant (Fig. 253).

There are two additional changes which occur as the endothelial cells fill with cytoplasm. One is a modification in their radial walls. The walls begin to form ingrowths, with those ingrowths toward the secretory face of the cells being larger than those toward the cortical side (Figs. 248, 255, 259-262). The other change is in the secretory material which becomes more finely reticulate (Fig. 264) and now appears relatively homogeneous (Figs. 247, 248, 264). In addition, the lipoidal moiety now appears to be secreted in two phases—a very dense core surrounded by less dense material (Figs. 264, 266, 273, 274, 279, 281).

In mature endothelium of post-anthesis styles, the cells are completely filled with very dense cytoplasm (Figs. 249, 250, 264). Nuclei of these cells are multinucleolate and lobed (Figs. 266, 271). The ER is once again rough, plastids no longer contain starch, and both plastids and mitochondria appear to be fewer in number (Figs. 262, 266). On the other hand, dictyosomes with many closely associated vesicles, microbodies, and polysomes become more numerous (Figs. 266-281). Wall ingrowths near the secretory face are now very large. The cell wall in these regions appears to have three phases (Fig. 277). The phases appear as gradations of wall fibril arrangement ranging from a normal, compact appearing wall, to loosely striated, to reticulate (Fig. 277). In one instance, a vesicle was seen to be half-fused with the plasmalemma surrounding an ingrowth (Figs. 268, 269).
Late stage  At anthesis, the stylar canal is completely filled with secretory material (Fig. 237) which is composed of three phases (Figs. 237, 249, 250, 280). Material in the center retains its affinity for protein stains, but now also stains positively for carbohydrates with basic fuchsin (Figs. 249, 250). The peripheral material also stains positively for both proteins and carbohydrates, but has a greater affinity for carbohydrate stain. Staining in the peripheral region, however, is somewhat spotty (Figs. 249, 250). Cuticular remnants are still present in some places, and other lipoidal materials which aggregate into ribbons or bands with an irregular outline seem to provide some measure of demarcation between the central and peripheral materials (Figs. 249, 250). Viewed with the SEM, the central material is rather coarsely granular while the peripheral substance has a smoother texture (Figs. 237, 280).

The dense osmiophilic bodies are still present in the lumen (Figs. 266, 279), but most are now surrounded by a less-dense substance. This less-dense substance also covers the secretory face of the endothelium and, in addition, is found by itself in the lumen (Figs. 266-281). Spherosomes, although greatly reduced in number, occasionally occur in the endothelium, but neither the very dense osmiophilic bodies nor the less-dense material around them, as seen in the canal, are found within the endothelial cells (Fig. 266).

Stigma

The stigma is recognizable shortly after the style begins to elongate from the carpels. This occurs well before anthesis. The first visible
evidence of stigmal differentiation is elongation of epithelial cells at the apex of the young style (Figs. 282-284). These cells continue to elongate and will eventually form individual papillae (Fig. 285) which collectively form the stigma (Fig. 286). At the time of anthesis, the papillae are fully extended but are not yet receptive for pollination. By approximately 24 hours after anthesis, the papillae secrete a small amount of material onto their surface, and only then are they receptive. A drop of nectar can be found at the base of the ovary at that time also (Figs. 293-295).

Cytoplasm in the papillae is dense and, except for the region around the nucleus, is restricted to a narrow band around a large central vacuole (Figs. 287-289). Nuclei may be lobed (Fig. 292) and are multinucleolate (Figs. 283, 288). ER and ribosomes are abundant (Fig. 292), but dictyosomes, mitochondria, microbodies, and plastids are sparse (Figs. 287-289, 292). Spherosomes are numerous and, as in the carpels and style, appear to become digested where they project into a vacuole while other spherosomes are homogeneous (Figs. 287, 288, 292).

Walls of papillae produce a characteristically rugose pattern externally (Figs. 285, 287), and internally the walls form small transfer wall ingrowths (Figs. 287, 288, 292). As the cells become actively secretory, the wall along the secretory face increases in width as the wall fibrils separate from one another to allow passage of the secretory material (Figs. 289-291). A cuticle surrounds each papilla and is noticeably thicker and more intensely staining than the cuticle covering epidermal cells of the style proper (Figs. 287-289).
**Post-pollination changes**

Two to three days after pollination, the tepals and the style show signs of withering. Tepals close over the gynoecium and begin to turn brown; the style shrinks and by the fourth or fifth day has collapsed and fallen to one side (Fig. 296). After about seven days, cellular degeneration is well-underway. Degeneration seems to occur in the endothelium first and then progresses centrifugally (Figs. 298, 299). Cell wall degeneration appears to progress bidirectionally from the center of a common wall toward the closest intercellular spaces (Figs. 298, 299). The very thick outer epidermal wall remains intact, but the cuticle is modified. Epicuticular wax disappears, the outer cuticle proper seems unchanged, and the inner cuticular layer is more electron transparent than it was previously (Fig. 297). Also, there are now some thin anticlinal fibrils in the inner cuticular layer which were not formerly discernible (Fig. 297). Remnants of the style and aborted ovules can still be seen after carpel dehiscence (Figs. 300-303).

**Ovules**

**Integuments**

Each ovule is invested by two integuments, an inner and an outer one. The inner integument is initiated before the outer, but both attain full size at about the same time. The first visual evidence of integument formation is the presence of a small buttress on the flank of the young ovules (Fig. 304). Integument initiation is coincident with archespore differentiation (Fig. 304), and is the result of anticlinal divisions in the nucellar epidermis (Fig. 305). Successive
anticlinal divisions (Fig. 306) cause the integument to grow around the nucellus, and, shortly after the inner integment nears the summit of the ovule (Fig. 307), the outer integument is initiated (Figs. 308, 309). The outer integument grows only around the side of the nucellus distal to the funiculus and thus does not completely surround the nucellus (Fig. 306).

Ovules begin turning anatropous at about the same time that the outer integument is initiated (Fig. 310). By the time ovules have turned approximately $135^\circ$, the outer integument has closely approximated the extent of the inner integument (Fig. 311). When ovules are turned a full $180^\circ$, both integuments are at their maximum extension, and the inner side of the outer integument is even with the outer side of the inner integument (Fig. 312).

The portion of the inner integument that surrounds the nucellus is generally two cells wide (Figs. 313, 314), but in some places it occasionally may be three cells in width (Fig. 313). That portion of the integument which forms the micropyle, however, is always at least three, and sometimes four cells wide (Figs. 312, 314). Cells of the two integuments are generally vacuolate (Figs. 312, 314) with relatively large nuclei that are usually found in a central, vice a peripheral location in the cells (Figs. 313, 315). The surfaces of the integuments and nucellus are covered by a cuticle, the epicuticular wax layer of which first appears as globules (Fig. 316). The globules later become floc-culent (Figs. 317, 318).
Nucellus

The nucellus is the first portion of the ovule to be initiated. At a very early stage one of the subdermal nucellar cells near the apex of the nucellus becomes noticeably different from its neighbors. This is the archesporial cell (Fig. 319). The cell (or cells) between epidermis and archesporial cell divide periclinally soon after the archesporial cell differentiates. By the time the archesporial cell has metamorphosed into the megaspore mother cell (MMC), the MMC lies several cells deep within the nucellus (Fig. 320).

As various additional changes that result in a sexually mature female gametophyte take place, the nucellus must undergo several coincident physiological and anatomical changes in order to produce a biologically functional system. These events will be considered in the following order: 1) changes to accommodate megagametophyte expansion, 2) starch influx, 3) changes in the nucellar epidermis and micropyle, and 4) hypos-tase formation. It must be kept in mind that these events are concurrent and are considered separately here only for the sake of clarity.

Changes to accommodate megagametophyte expansion The developing female gametophyte goes through two separate phases of expansion. The first phase begins with archesporial cell differentiation (Fig. 319) and terminates at meiosis when the MMC attains its maximum size (Fig. 320). The nucellus accommodates archespore and MMC expansion by a concurrent increase in size via mitotic divisions and cell expansion (Fig. 320). The developing gametophyte remains about the same size between meiotic prophase and the meiotic/mitotic interphase. Mitotic prophase heralds the beginning of the megagametophyte's second expansion phase.
As the functional megaspore enters mitotic prophase, it increases in both length and width. To accommodate the enlarging megaspore, the nucellus simultaneously undergoes growth and partial degeneration.

At the onset of mitotic prophase, cells bordering on the megaspore begin to degenerate and are later crushed as the megaspore expands. Nucellar cells between the developing megagametophyte and micropylar nucellar epidermis, the parietal cells, show signs of degeneration well in advance of physical contact with the megagametophyte (Figs. 321, 322). The first noticeable evidence of degeneration is an increase in nuclear density and staining intensity (Fig. 322). This is followed by a similar change in cytoplasmic density and staining intensity. The final phase of degeneration is cell collapse and resorption (Fig. 323). Nucellar cells in physical contact with, or one cell removed from, radial walls of the megagametophyte are either crushed or partially degenerated (Fig. 324). The same is true for parietal cells at the micropylar end of the megagametophyte. Degenerate cells in this region, however, may be two or three cells removed from the megagametophyte. This results in a large mass of degenerate cytoplasm and collapsed cell walls at its micropylar end (Fig. 325). Events occurring at the chalazal end will be considered later with the hypostase.

The time at which the expanding megagametophyte reaches the nucellar epidermis is variable relative to other events in the ovule. The earliest case noted was during interphase between the first and second mitotic divisions of the megaspore (Fig. 326). The most common condition, however, is for the megagametophyte to complete its axial expansion between the second and third mitotic divisions. During this interphase, the
megagametophyte generally attains its full length, and only remnants of the former parietal cells are seen between the megagametophyte and the inner tangential walls of the epidermis (Figs. 327-329). In none of the more than 300 ovules observed at the 8-nucleate stage were any intact parietal cells seen. Generally, by the time cell walls are formed between cells of the egg apparatus all traces of the former parietal cells are gone (Fig. 330, 331).

Starch movement During the interval between meiotic prophase I and prophase II, sugars move into the ovule at a rate sufficient to allow for accumulation and storage. By meiotic prophase II, amyloplasts are present in the chalazal region (Fig. 332), and usually by the end of the first mitotic division they are found in the micropylar region of the nucellus proper (Fig. 322). Amyloplasts are numerous around the megagametophyte and, as will be shown later, are most dense in and around the hypostase. Amyloplasts are not found in the nucellar epidermis (Figs. 330, 331, 333).

Nucellar epidermis The nucellar epidermis begins as a distinct, single layer of cuboidal cells (Fig. 319). By the time of MMC meiosis, epidermal cells at the future micropylar end (distal end) have elongated and become columnar (Fig. 320). Progressing toward the chalaza, epidermal cells are smaller until at the nucellus/inner integument junction they are the same size as the original epidermal cells of the archesporial stage (Figs. 320, 321).

The wall between the distal nucellus proper and nucellar epidermis is very distinctive. This wall is noticeably different as early as MMC meiosis. Already at this young stage it is thicker and more intensely
staining than walls of the nucellus proper. The difference is most noticeable at the distal end of the ovule (Figs. 306, 320), and the radial walls between epidermal cells of this end are also thicker and more intensely staining. Radial walls are thickest at their proximal end and taper distally (Figs. 306, 320). Both tangential and radial walls continue to thicken up to anthesis at which time they are at their maximum widths (Figs. 330, 331, 334). Thickening of the tangential wall is only on the epidermal side of the wall. In mature ovules, the central cell's wall is of normal thickness and does not contribute to the unique character of this boundary (Figs. 331, 333).

Staining the cells of this so-called nucellar cap for nucleic acids reveals large quantities of RNA and numerous small vacuoles (Fig. 333). Staining for protein shows the presence of many spherical and rod-shaped bodies (Fig. 331). Both staining reactions clearly show a large vacuole in the distal end of each cell (Figs. 331, 333). The cells have a large multinucleolate nucleus (Fig. 333).

Ultrastructurally, cells of the nucellar cap have large quantities of SER with swollen cisternae (Figs. 351-353). Microtubules are also prominent, and some of them appear to branch (Fig. 353). The most significant feature, however, is the presence of numerous vesicles which appear to be either pinching off of or fusing with the plasmalemma. Vesicles in contact with, or very close to, the plasmalemma and wall have contents similar in appearance to cell wall material. Contents of vesicles further away from the wall become progressively less dense and fibrillar or flocculent in appearance (Figs. 351-353). The number of these vesicles is greatest in the proximal end of each cell.
The micropyle is formed by the inner integument with the nucellar cap cells at the terminus (Fig. 335). It is at first lined only by the cuticle of these two tissues (Fig. 336). Both nucellar cap cells and inner integument cells facing the micropyle are secretory (Figs. 335, 337-339).

Secretory material is first seen during FM stage (Fig. 335) and appears to be mostly flocculent with some small condensed or coagulated regions (Figs. 338, 339). The micropyle becomes progressively filled with this substance (Figs. 340-344) which stains positively for protein and RNA but not for carbohydrate (Fig. 335). At anthesis, the micropyle is completely filled, and the exostomium is covered by a thin sheet of material (Fig. 345). This sheet is apparently ruptured by a penetrating pollen tube on its way to the egg apparatus.

Following fertilization, the micropylar material is still present but is now seen as a condensed mass around the distal end of the nucellar epidermis (Fig. 346). From an external view of mature seeds, the micropyle appears as an unobstructed opening in the testa (Fig. 347). Internally, however, the micropyle is still blocked by a plug of flocculent appearing material (Figs. 348, 349).

The first indication of hypostase formation occurs during the meiotic/mitotic interphase of the functional megaspore. Nucellar cells around the chalazal end of the functional megaspore become a bit more densely staining than their neighbors. Walls of some of these cells may show a
slight difference in their staining characteristics at this time also (Fig. 354). By completion of the first mitotic division of the megaspore, nucellar cells around the middle of the expanding megagametophyte show the same early signs of differentiating into hypostase cells (Fig. 356), and cells around the chalazal end of the sac are plainly evident as young hypostase cells (Figs. 355-357). By the four-nucleate megagametophyte stage, the hypostase is well-defined chalazally (Figs. 357, 358), but the full micropylar extent is not attained until the eight-nucleate stage (Fig. 359).

In functionally mature ovules, the hypostase extends axially from usually two, but sometimes only one (Fig. 359) or possibly as many as three (Fig. 357), cells beyond the chalazal end of the megagametophyte. In the opposite direction, it extends down to where the enlarged micropylar end of the megagametophyte meets the nucellar epidermis. Radially, the hypostase varies between one and three cells in width. In most ovules, the micropylar end is three cells wide (Fig. 359).

In approximately 25 percent of the ovules observed, cells immediately chalazad the hypostase proper undergo several periclinal divisions (Fig. 360). The result is an intercalary bloc of cells, in axial files of 4-6 cells in length, between the hypostase and the vascular strand in the chalaza (Figs. 361, 362). The daughter cells are cuboidal or rectangular, but other than being in discernible axial files they do not have any unique anatomical features (Figs. 363, 364).

Similar to conditions around the micropylar end of the megagametophyte, nucellar cells around the chalazal end are crushed during megagametophyte expansion as well (Figs. 365, 366). Expansion of the chalazal
end in both axial and radial planes is less than it is in the micropylar end of the megagametophyte. The increase at the chalazal end is generally limited to the one layer of cells that bordered the former megaspore. Expansion at the micropylar end is far more extensive. The result is a pyriform megagametophyte with a hypostase surrounding the narrow, necklike portion (Fig. 359).

In almost all of the eight-nucleate ovules observed, anywhere from one to three hypostase cells differ from all other hypostase cells. These unique cells are at or near the chalazal end of the megagametophyte, and they usually border on it. These cells are different in regard to their contents, and usually become distinct during the four-nucleate stage of megagametophyte development. Their cell walls seem to remain unchanged, but the cytoplasm becomes dense and several spherical bodies appear (Figs. 367-372). When these cells are fully metamorphosed, they contain thick strands of material and numerous dense spherical structures intermixed with the strands. There is no LM or SEM evidence for the presence of other cytoplasmic contents at this stage, including nuclei (Figs. 367-373). Both the strands and spheres stain very intensely for carbohydrate with basic fuchsin. The spheres have a general resemblance to amyloplasts when viewed by LM, but the same staining procedure used on both results in a different coloration.

When the megagametophyte reaches the point in development where it has attained its full size and characteristic pyriform shape, and the cells which comprise it are separated from one another by cell walls, starch grains are most numerous in and around the micropylar end of the hypostase (Fig. 359). Nucellar and hypostase cells surrounding the
antipodals have dense cytoplasm but relatively few amyloplasts (Fig. 375). As anthesis approaches, however, the chalazal end of the ovule and, in particular, the nucellar cells around the chalazal end of the hypostase fill with starch (Figs. 374, 376). The greatest proportion of starch reserves in mature ovules is in the chalazal end of the nucellus. At this stage in development, except for the 1-3 unique cells described previously, hypostase cells may contain some starch but they otherwise appear to be devoid of contents (Fig. 374).

**Funiculus and vasculature**

The funiculus is the stalk which attaches ovules to the placenta, and it is the funiculus that causes ovules to turn and become anatropous. The anatropous condition is the result of more mitotic divisions occurring on the distal side of the funiculus (Fig. 377). Vascular tissue which feeds the ovule appears during meiospore meiosis as a procambial strand (Figs. 378, 379). The procambium differentiates from parenchyma cells in the center of the funiculus.

A series of periclinal divisions in the procambium (Figs. 378, 379) causes both the procambial strand and the funiculus to increase approximately twofold in diameter. By the two-nucleate megaspore stage, most cell-division activity is completed, but an occasional mitotic figure can still be seen near the chalaza (Fig. 380). At this stage, procambial cells have elongated (Fig. 381) and, in some ovules, annular thickenings are being laid down (Figs. 382, 384, 385). Nuclei of differentiating xylem cells are multinucleolate and elongate to conform to the shape of the cells (Fig. 384). In mature ovules, the strand terminates within
the chalaza just above the hypostase (Figs. 383, 386).

**Archespore**

From the time ovules are first initiated up to when they are 4-5 cells long, all nucellar cells are of about the same size and appearance (Fig. 387). After this stage, one of the subdermal cells near the ovule apex begins to differentiate from its neighbors. In most ovules, there is one parietal cell between the young archesporial cell and nucellar epidermis. *Ornithogalum* ovules are thus crassinucellate (Figs. 388-393).

Archesporial cells increase in size to 2-3 times their initial volume. They all become somewhat elongate in shape but otherwise assume a variety of geometric configurations with angular contours (Figs. 389-393). The lateral contours become rounded as the archesporial cell continues to increase in size during its differentiation into the megsperme mother cell (MMC). Archespore/MMC transition thus produces a MMC that is circular in cross-section (Figs. 394, 395) and elliptical in longisection (Figs. 396, 397).

Staining ovules at the archesporial stage for protein reveals numerous circular and rod-shaped bodies. These bodies are found both in archespore and nucellus. The archesporial nucleus is multinucleolate with one very large nucleolus in or near the center of the nucleus. Smaller nucleoli generally occupy more peripheral positions (Figs. 389-391). Staining for nucleic acids reveals dense cytoplasm with large amounts of RNA. Also, numerous small vacuoles can be seen in both the archespore and nucellus (Figs. 392, 393).
Within the archespore, vacuoles seem to become smaller and cytoplasm takes on a more homogeneous appearance during the archespore/MMC transition. When the MMC is in early phases of meiosis, it no longer appears to have vacuoles. Its cytoplasm has a coarse but homogeneously granular appearance when stained for nucleic acids (Figs. 396, 397). Vacuoles seem to become larger in the nucellus (Figs. 394-396, 398), and the ovules have turned 90° from their original orientation (Figs. 398, 399).

In addition to these changes during the archespore/MMC transition, the parietal cell between archespore and nucellar epidermis divides once anticlinally. Following this division, the two daughter cells may divide either once (Fig. 398) or twice (Fig. 396) resulting in four or six parietal cells between the MMC and the epidermis.

In very young ovules, with the exception of nucellar epidermis walls, the archesporial cell wall stains more intensely than other nucellar walls (Figs. 390, 392). These staining differences are even more evident at the MMC stage (Figs. 394-396), particularly between nucellar epidermis and nucellus proper (Figs. 396, 398, 399).

Meiosis/Functional megaspore

Meiosis of the megaspore mother cell results in a linear tetrad of megaspores (Figs. 400, 401). Megaspores are separated from each other by cell walls that stain intensely with basic fuchsin. Results were negative from tests with aniline blue fluorescence for callose deposition on megaspore walls during meiosis.

Megaspores are unequal in size with the chalazal two being larger than the micropylar pair. Of the chalazal pair, the chalazalmost is
largest (Figs. 400, 401), and in most instances this megaspore develops into the female gametophyte. The nucleus of the chalazal megaspore is larger than that of the other three. It has a large central nucleolus plus one or more smaller nucleoli around the periphery. Nuclei of the other three megaspores are smaller, more dense, and stain more intensely (Figs. 400, 401).

Following telophase II and wall formation, the megaspores become vacuolate. The chalazal megaspore generally develops two vacuoles, one in either end of the cell (Figs. 400, 401). The other three megaspores may at first form several vacuoles, but the vacuoles eventually coalesce into a single large one (Figs. 400-402). The chalazal megaspore enlarges while the other three degenerate. The micropylar pair degenerates in advance of the micropylar megaspore of the chalazal pair (Figs. 402, 403).

The functional megaspore is generally elongate with the nucleus centrally located between the two vacuoles (Fig. 404). Not all functional megaspores have two vacuoles, however, and in those cells with only one it is usually in the micropylar end of the cell (Fig. 405). Staining the functional megaspore for protein reveals the presence of numerous circular and rod-shaped bodies, particularly around the nucleus (Fig. 404). During the meiosis/mitosis interphase, carbohydrates build up in the megaspore and, by the first mitotic division, amyloplasts are visible around the nucleus. Other organelles are also oriented around the nucleus in such a manner that they are equally distributed on both sides of the metaphase plate (Fig. 406).
Mitosis

The first mitotic division of the megaspore produces a binucleate megagametophyte with the two daughter nuclei at opposite poles. As the nuclei move to opposite poles, the cytoplasm which surrounds them becomes more dense and a large vacuole forms between them (Figs. 407-409). The nuclear division is not followed by cytokinesis and, therefore, a new wall common to the two new nuclei does not form between them. The wall of the former megaspore which does enclose the two nuclei, however, is thick and stains intensely (Figs. 407, 410).

Organelles (Fig. 410), particularly amyloplasts (Fig. 411), retain a perinuclear orientation as seen previously around the megaspore nucleus (Fig. 406). In most instances, amyloplasts are slightly smaller or equal in size to those found in the nucellus (Fig. 410). In some ovaries, however, the ovules have megagametophytes with amyloplasts that are much larger than those found in the nucellus. These very large amyloplasts often contain more than one starch grain (Fig. 411).

Four-nucleate megagametophytes are produced by a second set of nuclear divisions. Following these divisions, the resulting four nuclei are most commonly seen in a linear array of two pairs with one pair at each end of the megagametophyte (Fig. 412). Other nuclear patterns are found in which the nuclei from one pair (Fig. 413) or from both pairs of nuclei are located side by side (Fig. 414).

As in earlier stages, organelles are seen in a perinuclear arrangement (Figs. 412, 414, 415). In the four-nucleate stage, however, there is a differential distribution of organelles among nuclei. Organelles are equally distributed between nuclei of the chalazal pair (Figs. 412,
416), but in the micropylar pair, the micropylar nucleus retains the majority of organelles (Figs. 415, 416). And further, most of the organelles seem to be on the micropylar side of the micropylar nucleus (Figs. 412, 415, 517).

The third and final set of mitotic divisions produces the eight-nucleate megagametophyte which later becomes the sexually mature gametophyte. The divisions result in two polarized clusters of four nuclei each. Three of the four micropylar nuclei produce cell walls and form the egg apparatus which consists of two synergids and one egg (Figs. 418, 419). At the opposite pole, three of the four chalazal nuclei also produce walls and they form the three antipodals (Fig. 420). The two remaining nuclei, one from each cluster, are the polar nuclei (Fig. 420). They do not produce walls.

**Central cell**

A strand of cytoplasm extending from the egg apparatus traverses the central cell's vacuole (Figs. 418-421). This strand is usually seen as a narrow column of cytoplasm (Figs. 421, 422), but in some ovules it may be quite massive (Fig. 423). The polar nucleus from the micropylar cluster of nuclei is found within this strand (Figs. 422, 424). Staining the strand for protein reveals the presence of numerous circular and rod-shaped bodies, particularly around the nucleus (Fig. 424).

In *Ornithogalum*, the polar nucleus from the chalazal end of the megagametophyte does not move, but rather, it retains its chalazal position (Fig. 420). The micropylar nucleus, however, does move across the expanse of the central cell and joins its sister nucleus near the
antipodals (Figs. 424, 425). The central cell remains binucleate for only a short while as the two polar nuclei fuse soon after they juxtapose. Fused polar nuclei are referred to as the fusion nucleus or the secondary nucleus.

The central cell is the largest cell of the gametophyte. Most of its volume is occupied by a large central vacuole (Fig. 426), and its lateral walls are derived from the original megaspore. The terminal walls, however, are newly formed: the chalazal end by the antipodals (Fig. 420) and the micropylar end by the egg apparatus (Fig. 418). Cytoplasm is restricted to a very narrow band around the cell's periphery (Figs. 418, 424). Starch accumulates in the central cell during megagametophyte maturation and, at anthesis, numerous small amyloplasts are present in the cytoplasm (Figs. 427, 428).

Antipodals

The three antipodals occupy the narrow necklike chalazal portion of the megagametophyte (Fig. 420). The two micropylarmost antipodals, arranged one next to the other, occupy a central position between the chalazal end of the central cell and the third antipodal (Figs. 420, 425). The chalazal antipodal is the largest of the three and is vacuolate when first formed (Figs. 429, 432). The nucleus is suspended in the center of the cell and is surrounded by small vacuoles and other organelles (Fig. 431). The antipodals fill with cytoplasm and, at anthesis, they contain dense cytoplasm with numerous starch grains (Figs. 430, 433).
Egg apparatus

Egg  The three cells of the egg apparatus are arranged in a triangular fashion and occupy the micropylar end of the megagametophyte (Figs. 434-440). The egg is the smallest of the three cells and, in classical view, it appears to be perched atop the two synergids (Fig. 434). In actuality, however, the egg is in contact with the nucellar epidermis (Fig. 438) as well as the synergids. Only the chalazal portion of the egg projects above the synergids (Figs. 419, 436). A cell wall is not present between egg and central cell and is very thin between egg and synergids (Fig. 434).

A large vacuole in the micropylar end of the egg occupies most of the cell's volume while the greatest concentration of what little cytoplasm there is occurs around the nucleus. Cytoplasm is otherwise limited to a thin peripheral layer (Fig. 438). Even in young stages (Fig. 438), the nucleus is located in the chalazal end of the cell and, as the egg grows, its nucleus is typically raised to a level above the synergids (Figs. 434, 436).

Synergids

Cytoplasmic features  The two synergids are equal in size and appear as mirror images of each other when viewed either in median section (Figs. 434, 439) or externally (Fig. 440). In complement to the egg, the synergids each have a large vacuole in their chalazal end with the majority of cytoplasm in their micropylar end (Figs. 438, 439). The nucleus is usually centrally located in young cells (Fig. 441) and assumes a lateral position in mature cells (Figs. 434, 442). Synergid nucleoli typically have a very large pars morpha phase which gives them a
doughnutlike appearance (Figs. 434, 441, 442).

Both protein (Fig. 434) and nucleic acid (Fig. 443) stains show an intensity gradient in the synergidcs with the chalazal end of the cells staining more intensely than the micropylar end. Both types of stains also show that the cytoplasm is very dense, and, in addition with particularly protein stains, and it is coarsely granular. Staining tests for starch give negative results (Figs. 434, 443).

Ultrastructural examination reveals that plastids occur in clusters in the chalazal end of the synergidcs but none of them contain starch (Fig. 444). Plastids are not usually seen in the micropylar end of the cells. Mitochondria with large swollen cisternae are very abundant throughout the synergidcs (Figs. 445, 446).

Dictyosomes are also very abundant and most of them have numerous vesicles of various sizes associated with them (Figs. 445, 447, 448). Numerous dictyosomes and vesicles are present in the pockets of cytoplasm that are isolated by irregularities in the surface contour of the filiform apparatus (Figs. 449, 451). Vesicles of various sizes are found in wall material of the filiform apparatus (Figs. 449, 451, 453). Most of these vesicles contain some sort of fibrillar or flocculent material.

Synergidcs have large amounts of ER, the majority of which is SER (Figs. 447, 448, 452). Concentrations of ER are found around the periphery of the chalazal end of the cells (Fig. 452). ER in the micropylar end of the cells tends to be in short segments, and, in at least one instance, a segment of ER was seen to be in contact with the plasmalemma around the filiform apparatus (Fig. 446).
Other organelles found in synergids include numerous ribosomes and some microbodies (Figs. 445-447). In addition to the vesicles attached to or very closely associated with dictyosomes, there are a lot of other vesicles dispersed throughout the cytoplasm. Like their dictyosome-associated counterparts, the majority of these vesicles have some sort of electron-dense contents (Figs. 447, 448, 450).

**Filiform apparatus** Synergids are anatomically the most highly specialized cells of the female gametophyte by virtue of a massive proliferation of their micropylar cell wall, the filiform apparatus (FA) (Figs. 434, 442, 443). FA formation is one of the last anatomical changes to occur in synergid maturation. It does not begin to form until well after the common wall separating the two synergids is laid down (Figs. 439, 441). The FA first appears as a dense mass of darkly staining material along the micropylar wall of the synergids where the two cells meet the nucellus (Fig. 454).

The FA is medial along the plane perpendicular to the common wall between the two synergids (Figs. 434, 443). In the plane parallel to this wall, however, the FA is skewed to one side of the cells due to the vacuoles occupying the other side (Fig. 442). A large intercellular space is present at the base of the FA between its two halves and the nucellus (Fig. 443).

The FA has a very irregular surface outline with innumerable projections and protrusions of all shapes and sizes that extend into the synergid cytoplasm (Figs. 434, 456, 457). Material comprising the FA appears as a complex array of different phases that vary greatly in electron density (Figs. 446, 451, 453). The material stains intensely with basic
fuchsin for carbohydrates throughout the FA (Figs. 434, 443). It also stains throughout for the presence of protein (Fig. 434) and RNA (Fig. 443), but staining for these two classes of compounds is intense only around the FA's perimeter.

Viewed with the SEM, the FA can be seen to be surrounded by a vacuolate zone (Fig. 457). Vacuoles can also be seen around the FA with LM, particularly in cross-section (Fig. 442). This vacuolate zone is not universal over the entire FA (Fig. 456).

An additional anatomical feature unique to synergids is the presence of knobbed growth in the wall of each synergid out in the hook region (Figs. 434, 455). The knobs stain for the presence of carbohydrates but not for other classes of compounds.
DISCUSSION

The following discussion is divided into two basic sections. The first section follows closely the material presented in the results section and, in general, correlates the things I found in *Ornithogalum* with the information presented in the literature review. Some topics are treated in their entirety within the first section, while others will be considered again. The second section is more of a theoretical discussion of my ideas on some of the processes which must occur to eventuate sexual reproduction in angiosperms. Because of the extensive nature of the literature review, I felt it would be redundant to cite all the literature a second time, and so citations occur only where I thought a specific example was needed to punctuate a given point.

**Carpels, Style, Stigma, and Ovule Ontogeny**

Fusion of carpels and also of stylar segments during their ontogeny in *Ornithogalum caudatum* results in this species having an eusyncarpous gynoecium per the definition of Eames (1961). As is common amongst members of the Liliaceae (Eames, 1961; Sterling, 1972, 1973b,c, 1974a,b, 1975) and other monocot taxa, particularly palms (Eames, 1961; Uhl and Moore, 1971), the septal nectaries found in the ovary walls of *O. caudatum* are formed in part as a consequence of incomplete lateral fusion between adjacent carpels. Epidermal cells which come to line the nectary lumen become modified as secretory cells and produce nectar.

Similar to the situation in *Cocos* and *Ananas* (Uhl and Moore, 1971), the nectar in *O. caudatum* is exuded from a pore at the apical end of the nectary near the top of the ovary. Nectar runs down the outside
of the ovary wall and forms a small droplet in the axil of a subtending stamen or tepal. Secretory activity is correlated with anthesis and stigmal receptivity, being at its peak and causing nectar to exude when the stigma becomes receptive. Such a temporal correlation between secretory activity of nectaries and stigmal receptivity is interpreted as being one mechanism by which pollination is assured to occur at a time most favorable for successful pollen germination and fertilization.

Nectar within the nectary lumen of *O. caudatum* stains positively with aniline blue black and napthol black but not with basic fuchsin. These results indicate that the nectar consists of protein without any carbohydrate being present. A word of caution is due here, however, as it has long been known that nectar contains sugar. The negative result obtained from testing for carbohydrate most likely means that the sugar moiety is soluble in one of the reagents used during specimen preparation and was thus lost. Considering this factor, then, it seems safe to conclude that nectar produced by the septal nectaries of *O. caudatum* is a mixture of protein and carbohydrate, and possibly of other substances as well.

A detailed look at the changes which occur in cells which line the nectaries as they transform into secretory cells and a more specific analysis of the nectar would make an interesting study and a significant contribution to floral biology.

The elongate or palisadelike external carpellary epidermal cells of *Ornithogalum* are similar to those of *Polygonum* (Neubauer, 1971) and some palms (Uhl and Moore, 1971). They do not become sclerified, however, as do those in *Arachis* (Halliburton et al., 1975). The carpellary cuticle
of *Ornithogalum* is thick like that of some palms (Uhl and Moore, 1971), *Capsicum* (Munting, 1974), and *Lychnis* (Oostenink, 1976), and the outer cuticle proper exhibits a lamellar substructure as it does in vegetative organs of *Apium*, *Eryngium*, and *Humulus* (Chafe and Wardrop, 1973). A lamellate outer cuticle proper is not a ubiquitous feature, as evidenced by its absence in *Abutilon* and *Rumex* (Chafe and Wardrop, 1973). But, a character which does seem to be ubiquitous is the presence of a reticulum within the cuticular layer. It occurs in all five species just cited as well as in *Phorium* (Jarvis and Wardrop, 1974) and *Ornithogalum*. This reticulum is the structure which Chafe and Wardrop (1973) speculated to be involved with the secretion of cutin precursors.

An interesting feature of the cuticle on the gynoecium of *Ornithogalum* is that the epicuticular wax deposited on the ovary proper is not as dense as that along the style. Also, when viewed by SEM, the wax on the style is much more flocculent and gives the style a fuzzy appearance while the ovary appears almost smooth in comparison. In contrast to the differences noted for this layer, the cuticular layer and the cuticle proper appear to be identical in both regions of the gynoecium. If Hallam's (1970a,b) idea that lamellae of the cuticle proper are concerned with wax secretion, one might expect to see a difference in this layer, at least a quantitative difference, between ovary and style. But, no such difference was detected.

One can speculate from a morphological and anatomical standpoint that there is a physiological or functional reason for such a differential distribution of epicuticular wax. In that the style projects into space above the protective corolla and is vasculated closer to the surface
than is the ovary, the style is more subject to desiccating elements and presents a greater potential for water loss than does the ovary. Therefore, a thicker layer of wax along the style might help to reduce water loss through this organ in *Ornithogalum*.

In addition to the previously mentioned specialized secretory feature where it lines septal nectaries, the carpellary epidermis undergoes two further changes. Both of these changes occur post-anthesis and both of them are ultimately associated with the nutrition of the developing fruit and seeds.

The first is the development of partially sunken, aperigeneous (Fryns-Claessens and VanCotthem, 1973), anomocytic (Belyanina and Krylova, 1971) stomates shortly after the flower buds open. Stomata are a common feature of developing green fruits (Fischer, 1929; Klotz, 1975; Dave et al., 1975), and may even be supplanted by lenticels if the fruit becomes sclerified as in peanut (Smith, 1950). While restricted to the outer epidermis of the ovary proper in *Ornithogalum*, stomates in gynoecia of other Liliaceae (Vasil and Johri, 1964) and a variety of additional taxa (Fischer, 1929) may occur in the inner and outer epidermis of both ovary and style. Stomates of the inner epidermis, however, are nonfunctional. In gynoecia, sunken stomates are by no means unique to *Ornithogalum* as they are found in *Butia* (Uhl and Moore, 1971) and several other taxa (Fischer, 1929).

Stomatal development is necessarily a prerequisite in the development of functional chloroplasts and chlorenchyma. As in examples of chloroplasts from vegetative structures (Boardman et al., 1971; Cran and Possingham, 1974), chloroplasts in the ovaries of *Ornithogalum* seem to
develop from proplastids via a progressive proliferation of internal membranes to form a system of thylakoids. The young chloroplasts in *Omithogalum* ovaries have osmiophilic inclusions like those thought to be associated with membrane synthesis in chloroplasts of other plants (Greenwood et al., 1963). These inclusions seem to be catabolized in chloroplasts from older carpels. Although protein crystalline inclusions may occur within thylakoid systems in chloroplasts of some plants (Esau, 1975), no such crystals were found in *Omithogalum*.

The presence of chlorenchyma in green fruits allows the fruits to contribute to their own nutrition as well as to that of developing seeds, and considering the tremendous amount of metabolic activity going on within a developing fruit, the advantage of such a system is obvious to the point of requiring no further comment.

The other post-anthesis change to occur within the outer carpellary epidermis is the development of lipotubuloids and aggregations of spherosomes. Lipid materials appear to accumulate within epidermal cells shortly after the carpels become a deep green subsequent to chlorenchyma development. These fats may occur as single, large spherosomes, as an aggregation of small spherosomes, or as numerous small spherosomes within a lipotubuloid.

Similar accumulations of large quantities of lipid have been reported in liverworts, some dicots, several monocots, and even in algae and fungi. These accumulations have been described as occurring in different forms or phases, not only among the various taxa concerned, but within a given taxon as well (see Lipotubuloids and Spherosomes section of Literature Review). The latter condition, i.e., several forms within a given taxon,
suggests the possibility of an interrelationship between forms where a
given form represents one phase in a metabolic cycle.

According to Kwiatkowska (1966, 1971a,b), organelles and enzymes
necessary for lipid metabolism occur within a given lipotubuloid. By
incorporating $^3$H-palmitic acid into lipotubuloids of *Ornithogalum umbel-
latum* ovaries (Kwiatkowska, 1972a), she further was able to demonstrate
that the label appeared in lipid bodies outside the lipotubuloids. Con-
sidering these facts and the fact that in *O. caudatum* ovaries the lipid
materials occur in what are apparently three different phases, one is
led to a possible conclusion that the lipotubuloid represents a center
for lipid metabolism and that the other two phases probably represent
storage forms. It is possible that the very large individual spherosomes
form via an aggregation of several smaller ones. The reverse condition
is also a plausible course of events, i.e., the large spherosome being
the initial product and the aggregation of smaller spherosomes represent-
ing an early catabolic stage of the larger structure.

A temporal consideration of the appearance and disappearance of
lipotubuloids and extra-lipotubuloid spherosomes lends credence to the
idea that these entities represent a mechanism for storing photosynthate
produced by carpellary chlorenchyma. In both *Ornithogalum umbellatum*
(Kwiatkowska, 1966, 1971b) and *O. caudatum*, lipotubuloid formation and
lipid accumulation maximizes shortly after a functional chlorenchyma is
established, and disappears when the carpels lose their green color.
Since starch is not a prevalent storage form in the carpels, it seems
plausible to speculate that photosynthate produced in excess of immediate
need is transported to the epidermis, there converted to fat, and
ultimately stored in spherosomes. When photosynthate production levels drop as the chlorenchyma ceases to function, fat within the spherosomes is apparently hydrolyzed and transported to where it is needed for further metabolism. Maturing seeds with their developing elaiosomes and endosperm would provide such a metabolic sink.

Vascularization of *Ornithogalum caudatum* carpels does not exhibit any unique characters. The vasculature consists of five major bicallateral veins divided into three principal sets; one dorsal, two ventrals, and two laterals. A network of minor veins, most of which develop after anthesis, completes the carpellary vascular system. Procambialization is continuous and proceeds acropetally. This set of conditions seems to be common among other floral systems (Esau, 1942, 1943; Boke, 1949; Eames, 1961; Sampson and Kaplan, 1970), but great variety does exist in terms of vein number and distribution (Lersten and Wemple, 1966; Uhl and Moore, 1971), and also in terms of vein type and anatomy (Uhl and Moore, 1971; Kapoor, 1973).

The occurrence of taxonomically specific crystalloids within plastids of sieve tube elements has been reported for shoot and leaf tissue (Behnke, 1972, 1975; Behnke and Dahlgren, 1976), but to my knowledge they have not been described previously in floral tissues. According to the classification system of Behnke (1975), the crystalloids of sieve tube element plastids from *Ornithogalum caudatum* carpels are of the *Asarum* subtype of the monocotyledonous P-type with cuneate crystalloids. Such P-type plastids are defined as sieve element plastids that accumulate protein as a single product or in addition to starch (Behnke, 1975). Plastids of *O. caudatum* sieve tube elements do not accumulate starch,
but they do contain some small spherical structures 40-45 nm in diameter. Such structures are neither illustrated nor otherwise mentioned by Behnke, but they possibly represent the remains of the plastid lamellar system as suggested by H. T. Horner, Jr. (Iowa State University, personal communication, 1977).

The very dense and highly osmiophilic cells associated with the minor veins in the carpels are rather curious and are perplexing as to their true function. I have not encountered anything like them in the literature, and so can only speculate what their function might be. The tremendous number of mitochondria, each with many cristae and a very dense stroma, the very high ribosome population, and the lobed nucleus are features indicative of metabolically active cells. This, coupled with their association with the minor veins which differentiate as the carpels become photosynthetically active, and the fact that the cells become progressively more vacuolate as photosynthetic activity declines, suggest that these cells may play a role loading the phloem with photosynthetic product. The lack of a transfer-type wall structure in their walls, however, may tend to cast doubt on this possibility. But, considering that the occurrence of transfer-type walls is not a ubiquitous character of border parenchyma cells surrounding the minor veins of leaves, the possibility of their being cells which load the phloem becomes a bit more credulous.

As is typical of companion cell-sieve tube element connections (Robards, 1975), the plasmodesmata between these cell types in the carpels of Ornithogalum caudatum are enlarged on the sieve tube element side and are multibranched or ramified on the companion cell side.
Branched and very complex plasmodesmata occur also between epidermal cells of the style in *Ornithogalum*. They are ramified on both sides rather than expanded laterally as are the plasmodesmata found in the meristematic cells of dorsal angle meristems of *Selaginella willdenowii* (Wochok and Clayton, 1976).

A plenitudinous variety of different anatomies is encountered in the cortex of carpels. The differences mainly stem from the presence or absence of a given cell type, and the arrangement of that cell type in relation to other cells of the cortex. Sclerenchyma fibers, sclerified parenchyma, all types of sclereids, and crystalliferous idioblasts are the most common cell types in this regard while collenchyma, tube cells and cross cells, and others are more restricted in their occurrence (see Specialized Cells section of the Literature Review). *Ornithogalum caudatum* is no exception and contains two types of idioblasts, one of which is crystalliferous and the other is a new type not previously described.

Raphide crystals (presumably calcium oxalate) are the most common type of crystal to be found in floral tissues, but druse and crystal sand types are encountered also. The crystals of *O. caudatum* carpels are of the more common raphide type, but they have some unique anatomical and physiological features associated with them. They are unique in terms of the growth pattern of some mother cells, in terms of their being catabolized, and in terms of the chambers in which the individual crystals form.

Crystalliferous idioblasts are typically large cells and can be detected prior to crystal development by their large size and the large
size of their nucleus and nucleolus relative to neighboring cells (Horner and Whitmoyer, 1972). It appears to me that idioblasts tend to form while the organ in which they are developing is in a juvenile stage, and thus the large size of the idioblast can be compensated for during growth without distorting neighboring cells. There are no extant reports of crystalliferous idioblasts attaining their full size by growing intrusively into surrounding cells, but this appears to be the case in several instances in O. caudatum. Some examples were noted where adjacent cells were apparently invaded by an idioblast resulting in a syncytial crystalliferous idioblast formed from as many as five cells. The development of these apparent syncytia was not followed in detail during this study, and it is possible that this feature is an artifact. Such a study is needed to determine the validity of these observations.

It should be noted that crystals have only rarely been reported to be catabolized once they form. In O. caudatum, however, crystals within the carpels are catabolized and are completely gone by the time the carpels dehisce. The protein-carbohydrate matrix material in which the crystal chambers form, and the lamellae which comprise the crystal chambers persist for some time after the crystals disappear, but they too are eventually digested, as is all protoplasm of the carpels.

The character of principal interest with the raphides of Ornithogalum, however, concerns the lamellae and associated structures which form the chambers in which the individual crystals develop. Wattendorff (1976) reported that the chambers surrounding the hexagonal raphides in Agave leaves consist of lamellate sheaths which have curlicues at the small angles of the hexagon. According to Wattendorff, the lamellae do not
consist of polysaccharide, but rather are similar to suberinic cell wall layers of what he termed filling cork. He further stated that lamellar extensions which form curlicues offer the possibility of allowing interconnections to develop between sheaths. Wattendorff suggested that the lamellar structure could result from a decomposition of wax layers leaving behind the suberin lamellae.

The sheaths in *Ornithogalum caudatum* seem to be superficially similar in appearance to those of *Agave*. The sheaths in *Ornithogalum* are lamellate hexagons, and the lamellae from one side may overlap the lamellae from another side, particularly at the small angles of the hexagon. These extensions in *Ornithogalum* are shorter, less elaborate than those of *Agave*, and do not form curlicues. Also, in *Agave*, the extensions and curlicues are formed by lamellae from both sides of the angle, whereas in *Ornithogalum* only one side contributes.

No substructure was demonstrated for the lamellae in *Agave*, but in *Ornithogalum* it is seen that the lamellae are composed of chains of spheres $61 \times 10^3$ in diameter. Other features demonstrated in *Ornithogalum* but not in *Agave* are the abrupt terminations and/or anastamoses of lamellae, the variable number of lamellae between separate chambers and along different radii of a given chamber, and the different appearance of the innermost lamella which seems to be continuous around the entire chamber.

The slime body in *Ornithogalum* stains positively for protein, carbohydrate, and RNA. The crystal sheaths stain positively with basic fuchsin for carbohydrate, and with azure B, they stain a rose to red-violet color indicating the presence of mucopolysaccharides. These results are
contrary to those of Wattendorff (1976) for the sheaths in *Agave*. Wattendorff admits, however, that fixation of his material was not adequate and that negative results with the Thiéry reaction may be a consequence of this poor fixation.

In *Agave*, the slime body has a reticulate ultrastructure, but in *Ornithogalum*, it has a fibrillar and somewhat flocculent appearance and it contains circular and tubular components 54 Å in diameter. The circular structures most likely represent tubules seen in cross-section. The presence of RNA and protein within the slime tenders the possibility that synthetic reactions occur within this material, and the similarity in size and appearance of these tubules and the tubules of the sheaths is suggestive that sheaths may form, at least in part, from matrix tubules. Based on the information available, then, it seems plausible to conclude that the sheaths are composed of materials which come from the slime bodies, and that the tubules, which are randomly oriented in the slime, become organized into parallel rows of a regular periodicity to form the intralamellar and the interlamellar tubules.

This hypothesis is certainly conjectural, being based as it is only on data from static images of mature structures. A detailed look at the ontogeny of the idioblast proper, the slime body, and the crystal chambers is needed either to verify or to discredit the hypothesis.

In addition to crystalliferous idioblasts, *Ornithogalum caudatum* carpels contain another type of idioblast, which, to my knowledge, has not been described previously. These cells begin to appear well before anthesis, and, although they are eventually found throughout the carpels, they occur most abundantly around the lateral veins, septal nectaries,
and suture zones. The idioblastic material reacts positively with stains for carbohydrate and for protein. Following anthesis the morphology of this material changes, becoming progressively more diffuse. By the later stages of seed development, it has disappeared.

The true physiological role of this material is unknown, but it might represent a storage form of substances used by the septal nectaries for the synthesis of nectar. The proximity of the idioblasts to the nectaries, the incidence of idioblast differentiation just prior to the initial appearance of secretory material in the lumen of the nectaries, and the apparent onset of degradation of the idioblasts following the peak of nectary activity are factors which suggest and support this interpretation. An autoradiographic study would be useful to determine if this is the case.

Following anthesis, a few cells of the carpellary cortex were noted to form transfer-type wall ingrowths, and in a few other instances paramural bodies were found. Paramural bodies, defined as membranes external to the plasmalemma (Marchant and Robards, 1968), are of two types. Lomasomes are formed by membranes of cytoplasmic origin while plasmalemmasomes are membranes of plasmalemma origin (Marchant and Robards, 1968; Heath and Greenwood, 1970). In that the membranes within the paramural bodies of Ornithogalum have the same substructure as the limiting membrane of nearby cytoplasmic vesicles, these paramural bodies are apparently of the lomasome type. In general, however, cortical cells of the carpels are not anatomically modified for transport of large quantities of material.
The endothelium, or adaxial epidermis, of the carpels forms the layer which lines the locules. The anatomy and the physiological role of this layer varies greatly among different taxa. It may degenerate completely at a very early stage, it may become secretory, or it may proliferate into several layers. In *Ornithogalum*, the endothelium is not secretory and undergoes anticlinal divisions only, but it does become anatomically modified. As the time for dehiscence approaches, the inner tangential wall thickens substantially. With this one greatly thickened wall, the endothelium resembles the annulus of a fern sporangium, and quite possibly functions in an analogous manner which causes the carpels to split along their septa at dehiscence.

Gynoecia are classically divided into three morphological sections: stigma, style, and ovary. As research into the role played by each of these parts continues, it is becoming more evident that, in addition to providing a surface for pollen tubes to grow along (or through), each section plays a significantly different role in insuring that the one common goal, sexual reproduction, can take place successfully.

Morphologically, the tissue on or through which a pollen tube must grow to reach an ovule is the same from the stigma to the base of the locule. In all cases, this tissue is either the adaxial epidermis of the carpel or its immediate derivative. In some cases this tissue has become greatly modified along its entire length and in other cases only a portion has become significantly modified, while in yet other examples little, if any, modification has taken place.

The obturator seems to be somewhat of a "bastard child" with some authors claiming that it originates from the funiculus, some claiming it
originates from the placenta, and yet others propose its origin to be
the integuments. Some say it constitutes a portion of the transmitting
tissue, while others look on it as a separate entity. In short, nobody
agrees on a common definition. This apparent disagreement among different
investigators seems to be the result of a diverse array of gynoecial
anatomies with each investigator considering only the anatomy of the
plant being scrutinized at the time.

In light of this confusion and the lack of a concise set of defini-
tions, I have formulated the following set which I tender for considera-
tion. The definitions are based on the fact these structures or areas
are actually of the same morphological origin, as noted previously, and
that there is at least one common function among them.

Transmitting tissue: That portion of the gynoecium which serves as a
landing and germinating surface for pollen grains, and any tissue along
or through which pollen tubes grow until they enter a micropyle. This
tissue extends from the stigma to the base of the locules, and consists
entirely of carpellary adaxial epidermis, or its immediate derivatives.
Transmitting tissue may be variously modified according to its function(s)
and its location in the gynoecium.

Stigmatic transmitting tissue: The epidermal layer of the stigma
which serves as a landing and germinating surface for pollen grains.
May be covered by an exudate. Some recognition and/or incompatibil-
ity reactions occur here. Often papillose.

Stylar transmitting tissue: That portion of the transmitting tissue
which occurs in the style. May consist of a single epidermal layer
lining the stylar canal (hollow styles) or of several layers filling
the canal (solid styles). Intermediate conditions are also found.
Some recognition and/or incompatibility reactions occur here. With
very few exceptions, is universally secretory. Provides nutritive,
and possibly chemotropic, materials for pollen tube growth. Various
anatomical modifications possible.

Ovarian transmitting tissue: That portion of the transmitting
tissue which occurs in the ovary. It may be secretory and may
provide nutritive, and possibly chemotropic, materials for pollen tube growth. Various anatomical modifications, termed obturators, are possible, or it may be unmodified.

**Obturator:** Any of several modifications of ovarian transmitting tissue classified according to origin, viz., placental, funiclar, integumentary, or a combination thereof. Are usually secretory. May provide surface onto which micropylar exostome opens (placental, funicular, and placental-funicular only) or may fill micropyle to varying degrees (all types). May appear as a pad or swelling (placental, funicular, and placental-funicular only) or as hairs or filaments, or tufts thereof (all types). Are most prevalent in Euphorbiaceae, Rosaceae, and Liliaceae, but are found in many other taxa as well.

In *Ornithogalum*, the obturator develops from a proliferation of tissue at the base of the funiculus and from the tip of each carpel margin, and is thus of the placental-funicular type. When mature, the obturator forms a pad of tissue which extends axially almost the entire length of the locule, and horizontally all the way across the two rows of ovules. Thus, the base of each funiculus becomes completely surrounded by obturator and the entire central column of tissue in the ovary provides a surface conducive to pollen tube growth. In that the obturator in *Ornithogalum* is secretory, this entire surface is coated with material which most likely provides pollen tubes with nourishment and it may also contribute to directing pollen tube growth, via some sort of chemotropic mechanism. These ideas and a greater consideration of the role of obturators will be discussed more fully in a separate section of pollen tube growth.

Epidermal cells of the style have several interesting features, not the least of which is their outer wall which becomes so greatly thickened and mantled by a very thick cuticle. It is interesting, too, that only
the outer layers of this wall become folded to form the rugae which characterize their mature anatomy. Perhaps this thickening provides the means by which the style supports itself in that the style has only a minimal amount of vascular tissue and is otherwise devoid of any mechanical tissue.

Although abundant spherosomes are found in the stylar epidermis, none attain the grandiose size of those in the carpellary epidermis, and no lipotubuloids are found in the style. There are numerous cytoplasmic filaments which traverse the large central vacuole indicating that there is probably a good deal of cytoplasm-vacuole exchange required by whatever the metabolic processes are that these cells specialize in. Considering the abundance of lipid material found in the epidermis of the style, and of the ovary as well, it seems likely that this large cytoplasm-vacuole surface area is somehow related to the lipid metabolism in these cells.

Literature on styles seems to be aimed in three principal directions: (1) morphological interpretations, (2) physiological functions, and (3) anatomy of the transmitting tissue. Very little has been written about the anatomy of the cortical tissue, particularly at the ultrastructural level. In *Ornithogalum*, as in most styles observed, the cortex consists only of elongate parenchyma cells. Many of these cells in *Ornithogalum* exhibit secondary vacuolation with the secondary vacuoles being formed from the tonoplast. This contrasts the situation, albeit for nonstylar tissue, described in a series of papers by Mahlberg and co-workers (Mahlberg et al., 1970, 1971, 1974; Mahlberg, 1972) in which the secondary vacuoles are reported to originate from the plasmalemma.
According to Mahlberg et al. (1974), the plasmalemma may invaginate into the central vacuole but such invaginations do not fuse with the tonoplast (Mahlberg et al., 1974). In *Ornithogalum*, the plasmalemma seems to be involved with secondary vacuolation in the cortex of the ovarian portion of the carpels, but not in the stylar portion. Secondary vacuoles in the style appear to be entirely tonoplast-bound entities which may contain ER and other cytoplasmic components that have a fibrillar to flocculent appearance. Secondary vacuolation may represent a mechanism for isolating this particular type of material, a function commonly attributed to vacuoles in general (Dainty, 1968).

Vasculation of the style in *Ornithogalum* is of the type most frequently encountered, i.e., a continuation of the dorsal vein from the subtending carpel. This condition is also seen in some other members of the Liliaceae (Sterling, 1974b) and contrasts an alternative situation in which the ventrals also extend into the style as in some Palmae (Uhl and Moore, 1971) and some Cunoniaceae (Dickison, 1975). Morphologically, the style in *Ornithogalum* offers no unique features such as the two 90° turns found in the style of peanut (Smith, 1950), or the greatly reduced sterile style of some of the flowers within a given raceme of *Solanum mammosum* (Martin, 1972). The style in *Ornithogalum* is long and narrow, a type of morphology which may serve to screen out incompatible pollen tubes as suggested by Whitehouse (1950).

The activity of a basal intercalary meristem during early growth produces most of the stylar cells in *Ornithogalum*, as it does in *Vinca* (Boke, 1949) and *Petunia* (Linskens, 1974b), with the balance of growth resulting from cell elongation. This condition contrasts with that of
**Datura** (Satina, 1944) where meristematic activity is continual until mature proportions are attained, and with that of corn and buffalo grass (Jones and Newell, 1948) where the style continues to elongate until pollinated. The condition of gynoecial maturation proceeding basipetally as in the Palmae (Uhl and Moore, 1971) seems to be rare and is possibly confined to this taxon. Of the limited experimental work done on stylar development, the castration experiments with *Petunia* by Linskens (1974b) are the most interesting. That he was unable to induce normal stylar development with presently known growth regulators, following castration performed during specific developmental stages, would tend to indicate that some control over flower development is exerted by anthers by way of an unidentified growth regulator synthesized within the anthers. Once this substance is characterized, it would be interesting to apply it to the stunted styles of *Solanum mammosum* to see its effect on the growth of these dwarf styles.

As noted by Hedwig (1793), stylar transmitting tissue may be more developed than ovarian transmitting tissue, or vice versa, depending on the taxon. Variation in the extent of development within the style is the basis for Hanf's (1935) classification scheme of solid, half-solid, and hollow styles. Satina (1944), by inducing chimeras in the styles of *Daucus*, was able to demonstrate that the tissue which fills the center of the style in solid and half-solid styles is either adaxial epidermis proper or its immediate derivative.

In the case of *Ornithogalum*, the stylar transmitting tissue is less developed than the ovarian transmitting tissue in the sense that *Ornithogalum* has a hollow style lined by only one layer of transmitting
tissue cells whereas the ovarian transmitting tissue proliferates to form a prominent obturator. The transmitting tissue in both regions of the gynoecium is secretory, and in the sense of secretory activity, stylar transmitting tissue is much more developed than is the ovarian transmitting tissue. No chloroplasts are found in the transmitting tissue of Ornithogalum as they are in tobacco (Bell and Hicks, 1976). But, as in some other members of the Liliaceae (Vasil'ev, 1970; Rosen and Thomas, 1970), Ornithogalum does have amyloplasts in the stylar transmitting tissue, and so does tobacco (Bell and Hicks, 1976), Lycopersicon (Cresti et al., 1976), cotton (Gore, 1932; Jensen and Fisher, 1969), and several others (Vasil and Johri, 1964; Green, 1894). Lipids are found in the stylar transmitting tissue of some lilies (Vasil'ev, 1970) and in Ornithogalum, but Ornithogalum is not among the few genera in which crystals occur in this tissue. In only one instance in one individual plant of Ornithogalum was a hairlike protrusion seen projecting into the stylar canal, but such outgrowths are common in some other members of the Liliaceae (Wunderlich, 1937).

In taxa with solid styles, plasmodesmata usually disappear from the lateral walls of the transmitting tissue cells (Sassen, 1974; Cresti et al., 1976; Bell and Hicks, 1976). Capsella (Sassen, 1974) is a noted exception. In Ornithogalum and other taxa with hollow styles, however, they remain intact. This difference can probably be attributed to the difference in the way material is secreted in the different types of styles. In solid styles, transmitting tissue cells secrete material onto all of their lateral walls so that the cells form columns of cells virtually isolated from one another by this secretory material. In
hollow styles, however, only the wall which lines the stylar canal is secretory. These differences allow adjoining cells to retain their plasmodesmatal interconnections in hollow styles but not in solid styles where they become separated laterally by secretory material. Plasmodesmata are ever-present in the vertical end walls of transmitting tissue cells of all types of styles.

In regard to functioning as secretory cells, transmitting tissue cells lining the stylar canal of Ornithogalum, some other members of the lily family (Vasil'ev, 1970; Rosen and Thomas, 1970; Dashek et al., 1971) and alfalfa (Johnson et al., 1975) have modified cell walls to enhance transport of the secretory material out of the cells. With the exception of Ornithogalum, the centripetal wall of these cells forms transfer-type ingrowths, which together may be referred to as a Wandlabrinthe. In Ornithogalum, however, the cell wall facing the canal shows no such modifications, but the anticlinal walls form very large ingrowths at a distance from the canal of approximately one-third the length of the wall. These ingrowths in Ornithogalum appear to be formed by a localized expansion or stretching of the existing wall and thus do not have the same appearance as those of a Wandlabrinthe which are formed by a localized proliferation of additional wall material.

The lack of a Wandlabrinthe along the internal surface of the secretory face but the presence of heavy deposits of what are apparently lipoidal materials along the exterior surface, and the presence of large regions of expanded cell wall along the radial walls with visible evidence of material being extruded into the expanded region by way of vesicles fusing with the plasmalemma suggests that the different moities
(at least three) comprising the material in the canal are released from the cells in different ways. Materials of direct plastid origin most likely move through the centripetal wall whereas those of dictyosomal, and possibly of ER origin, probably exit via emiocytosis (reverse pinocytosis) in the expanded wall regions.

According to Sassen's (1974) report, stylar exudate seems to be universally similar, consisting of polysaccharide and protein. The occurrence of lipids was not mentioned by Sassen (1974), but Cresti et al. (1976) noted its absence in *Lycopersicon*. Material filling the stylar canal in *Ornithogalum*, however, is cytochemically recognizable as being composed of lipid, protein, and carbohydrate. Analogous to the situation in *Lycopersicon* (Cresti et al., 1976) where secretory activity seems to occur in two phases, the secretory activity in *Ornithogalum* occurs in three phases.

In both cases, each phase is marked by the predominant production of one of the fractions which comprises the exudate, and by a high population of the organelles associated with the synthesis of that type of compound. For example, in *Lycopersicon*, the polysaccharide moiety is produced first. Protein synthesis follows, subsequent to a massive increase in the amount of RER and polysomes in the endothelial cells (Cresti et al., 1976). ER is also an important factor in exudate production in *Petunia*. In *Ornithogalum*, lipid and protein appear in the first phase, a second type of lipid denotes the second phase, and carbohydrate production marks the third phase.

The first secretory activity immediately follows sloughing of the cuticle from endothelial cells and occurs well before anthesis. This
timing contrasts with *Lilium* (Rosen and Thomas, 1970; Dashek et al., 1971) in which the extrusion of exudate into the style does not occur until three days after anthesis. When viewed with light optics, the initial secretory substance in *Ornithogalum* has a smooth to flocculent appearance, but electron optics resolve it to be composed of a reticulum of fibrillar material. Considering the staining reactions noted previously, the fibrils comprising this reticulum are most likely a lipoprotein of some sort.

Prior to sloughing of the cuticle and the initial secretory phase, the endothelial cells of *Ornithogalum* contain numerous spherosomes, but these disappear rapidly once the cuticle is sloughed and secretion begins. This rapid disappearance of the spherosomes indicates that they served at least as a partial source for the lipid being secreted. As one would expect for cells synthesizing protein and lipid, ER and ribosomes are the dominant organelles in the endothelial cells at this stage of development. Some microbodies are also present, but not to any great extent. Although these microbodies might possibly represent glyoxysomes, the large amount of lipid metabolism which takes place in these cells suggests that the few microbodies are either exceptionally busy bodies or that they do not have a significant role in the overall lipid metabolism which occurs in the endothelium.

During the initial burst of secretory activity, endothelial cells undergo a transition in which they gain a considerable amount of cytoplasm. The ER predominantly changes from rough to smooth, and plastids and mitochondria increase in frequency. The plastids contain starch, have a very dense stroma, and are surrounded by SER. These changes and
the appearance of the plastids are very similar to the condition reported for the styles of *Lilium regale* and *L. davidii* (Vasil'ev, 1970) as they approach anthesis. Once the endothelial cells have assumed this new appearance, the second secretory phase is underway and a noticeable increase in the amount of globular lipid material being secreted occurs in the canal.

This increased incidence of lipid secretion is not unexpected when the appearance of the plastids and their close association with SER is taken into account. These two organelles seem to be almost ubiquitously involved in the synthesis of lipoidal exudates, and ER ensheathed plastids have been reported in numerous examples of lipid and resin glands (Wooding and Northcote, 1965; Schnepf, 1969a,b,c; Dumas 1974c,d; Tsekos, 1974; Dell and McComb, 1974; Fahn and Evert, 1974; Fahn and Benayoun, 1976; H. T. Horner, Jr., Iowa State University, personal communication, 1977). Many of these authors implicate both of the organelles as being involved in the secretory phase, and Dumas (1974c) speculates that the ER may also provide a means for rapid transport of the product away from the site of synthesis.

Although examples are known where plastids alone are involved in lipid synthesis, viz., *Viscaria* (Tsekos and Schnepf, 1974), and where ER alone is involved, viz., *Petunia* (Kroh, 1967), *Forsythia* (Dumas, 1973a), and *Citrus* (Thomson et al., 1976), they do most commonly occur together. It seems reasonable, then, to support the hypothesis that both organelles are directly involved in the synthesis of the lipid exudate. Lipoidal exudates generally are not recognizable within the mother cell, and so, as postulated by H. T. Horner, Jr. (Iowa State University, personal
communication, 1977), the product is apparently synthesized and trans­
ported out of the mother cell in a form that is different from the final
form it assumes once it gets outside of the cell. That ER functions
secondarily in this transport of plastid-ER synthesized lipid (Dumas,
1974c) seems to be a plausible idea.

Carbohydrate material is not detectable in the stylar exudate until
the third phase of the secretory cycle. During this phase, numerous
very large dictyosomes appear in the endothelial cells, and the type
of plastids so prevalent in the second phase become relatively incon-
spicuous. ER once again becomes smooth, and polysomes and microbodies
become more numerous. Anthesis occurs during the third phase, and mito-
chondria start to degenerate during its latter stages. Mitochondrial
degeneration is the process which probably heralds the end of secretory
activity and marks the beginning of endothelial degradation.

The polysaccharides found as part of the exudate in other stylar
systems are mainly pectins (Rosen and Thomas, 1970; Kroh, 1973, Sassen,
1974) and, although specific tests for pectins were not performed in
Ornithogalum, there is no reason not to assume that the bulk of the
carbohydrate material in Ornithogalum styles is pectinaceous also. It
seems logical to correlate the appearance of dictyosomes and the change
in ER with the synthesis and secretion of this material in Ornithogalum.
Mucilage and nectar glands in general seem to be dominated by ER and/or
dictyosomes (Horner and Lersten, 1968; Schnepf, 1972; Schnepf and Fross,
1976; Rachmilevitz and Fahn, 1973; Kristen, 1974, 1975; Benner and
Schnepf, 1975; Fahn and Rachmilevitz, 1975; Heinrich, 1975), and the
synthesis and intracellular transport of the exudate in these cells has
been attributed to these two organelles (Horner and Lersten, 1968; Rachmilevitz and Fahn, 1973; Kristen, 1974; Benner and Schnepf, 1975).

As mentioned in passing earlier, the actual extrusion of material into the stylar canal in Ornithogalum probably occurs by two different mechanisms. No pores as in the lipid producing glands of Cannabis (De Pasquale, 1974) or plasmodesmata, like those leading to schizogeneously formed oil ducts in Rhus (Fahn and Evert, 1974), were found in the secretory face of endothelial cells. Thus in Ornithogalum, the lipid and protein, and possibly some carbohydrate fractions, are probably carried across the plasmalemma along the secretory face by an active transport mechanism, and then move through the wall proper into the canal. Other materials, most likely carbohydrate in nature, are first extruded into the large expanded regions of the radial walls, the development of which coincides with dictyosome appearance. Extrusion in these regions seems to be by way of dictyosome or ER vesicles fusing with and, then, passing through the plasmalemma. The loose nature of the wall fibrils in these regions might then allow movement of the material into the canal.

Like the stigmas of most entomophilous flowers (Eames, 1961), the stigma of Ornithogalum is small and simple. It consists of only a single layer of papillate epidermal cells, and it is globose in its general outline. Looking straight down onto the top of the stigma reveals that it is slightly lobed with each lobe corresponding to a subtending carpel as noted for other systems (Satina, 1944; Bell and Hicks, 1976). The stigmatic papillae of Ornithogalum are vacuolate and have dense cytoplasm with large nuclei as they do in many other taxa (Maruyama et al., 1962;
Rosen and Thomas, 1970). The large nucleus in these cells may be due to endomitosis, a condition noted to occur in *Spironema* (Tschermak-Woess, 1959). *Ornithogalum* is the only plant in which transfer-type wall ingrowths have been reported to occur.

Some stigmas, e.g., *Narcissus* (Chen, 1971) *Brassica* (Roggen, 1972), and *Ornithogalum*, have a prominent cuticular layer covering the stigmatic papillae, while others, e.g., sweet potato (Martin and Ortiz, 1967), do not. If one accepts the tenet of Roggen (1972) that the wax layer represents the incompatibility layer by virtue of its allowing or disallowing pollen grains to adhere to a dry stigma such as that of *Brassica*, then in this type of plant the incompatibility reaction hinges upon a pollen grain being able to adhere or not adhere to the stigma. This really does not seem to be a feasible explanation in that the very morphology of the stigma in this type of plant is one that enhances the lodging of pollen grains between its papillae. The cuticle of dry stigmas might participate in the overall incompatibility reaction (used in its broadest sense) of the plant, however, by helping to maintain certain moisture levels on the stigmatic surface or by containing a substance which causes a reaction inhibiting pollen grain germination that only pollen from the appropriate source is capable of blocking.

The origin of integuments has been a subject of morphological debate for some time. Historically, Schleiden (1837) suggested that integuments originate in the chalaza, while contemporarily, Bor and Kapil (1976) think integuments—or at least the outer one—originate from the funiculus. The evidence presented in this dissertation shows that the integuments in *Ornithogalum* arise from the nucellar epidermis while the ovule is still
in a young stage. Morphologically, then, transmitting tissue and integuments are both derived from the same tissue, and the integuments represent nothing more than specialized folds in the adaxial carpellary epidermis which now invest the megasporangium. Thus, the contention of Boesewinkel and Bouman (1967), Heel (1970), Heel and Bouman (1972), and Rembert (1977) that integuments originated from a fusion of parts does not seem to be a reasonable hypothesis.

The view proposed by Schleiden (1839), Eames (1961), and Fahn (1974) that the absence of an outer integument on the funicular side of bitemporal anatropous ovules is the result of congenital fusion is, however, a reasonable hypothesis and is supported by cross-sectional views of the ovules of Ornithogalum. In that both integuments are derived from the same tissue they are homologous, and thus the contention that the concept of congenital fusion should be disregarded because the inner and outer integuments are not homologous (Bor and Kapil, 1976) should itself be disregarded. The results of this congenital fusion in Ornithogalum can be seen as the much greater thickness of the funiculus on the ovular side of the funicular vascular tissue than on the side of the funiculus opposite the ovule.

The timing of integument initiation in Ornithogalum is slightly earlier than in most ovules, but it should be noted that the entire schedule and sequence of events which occurs during ovule maturation is seemingly as variable as there are different taxa. This great variability ultimately ends up with the same functional end product and so such temporal considerations, with a few exceptions to be noted later, are moot at best.
Integuments seem to have several basic functions which include protection of the internal structures from desiccation and mechanical injury, formation of a micropyle, and formation of a seed coat. Other characters of variable taxonomic distribution include: forming integumentary tapeta, serving as food storage areas; obturator, elaiosome, and caruncle formation; and the formation of various hairs, spines, and like structures that aid in mechanical dispersal of the seed. Some, although rarely, may even contribute photosynthetic products.

The integuments of *Ornithogalum* are covered by a cuticle and are thus well suited to help prevent water loss (it should be remembered that locules are not fluid-filled), and at seed maturity they form an elaiosome indicating that *Ornithogalum* is myrmecochorous. The integuments of *Quercus* (Mogensen, 1973) and *Tricyrtis* (Ikeda, 1902) store the greatest concentration of food reserves within the ovules, but, as in *Agave* (Tilton, 1974), the integuments of *Ornithogalum* do not have a significant role as storage organs. The importance of integuments as storage organs in a given taxon may be related to their vasculature (Tilton, 1974). For example, the outer integument of *Quercus* is vasculated (Mogensen, 1973) whereas vascular tissue does not occur in the integuments of *Agave* (Tilton, 1974) or of *Ornithogalum*.

Formation of the micropyle is, of course, a significant contribution of the integuments to the functioning of the ovule. In *Ornithogalum*, the integuments contribute structurally and physiologically to ovule function through their contribution to the micropyle, but this topic is discussed in a later section.
By virtue of the MMC being located several cells deep within the nucellus, ovules of *Ornithogalum caudatum* are classified as being crassinucellate, as are those of over half of the families surveyed by Davis (1966). This character is generic in the Liliaceae (Davis, 1966), and, in addition to *O. caudatum*, *O. gussonei* and *O. umbellatum* (Czapak, 1972), have been shown to be crassinucellar.

Growth of the nucellus occurs by both anticlinal and paraclinal divisions in *Ornithogalum*, and it maintains pace with expansion of the archespor and MMC. During expansion of the female gametophyte, however, cells surrounding the megagametophyte undergo degeneration, particularly on the micropylar end. Nucelli in some ovules, viz., *Agrostemma* (Cook, 1903b), *Hordeum* (Norstog, 1974), and *Diplotaxis* (Pacini et al., 1975), are apparently characterized by two cell types or zones. One cell type is generally very thin-walled or otherwise unstable in appearance, and occupies the area into which the megagametophyte will eventually expand. The other cell type has a stouter appearance and persists until the entire nucellus degenerates. *Ornithogalum*, however, exhibits no such zonation.

Norstog (1974) postulated that nucellar cells, which degenerate and form what he terms the nucellar lysate in *Hordeum* in order to accommodate the growing megagametophyte, do so not as a result of mechanical pressure. This also seems to be the case, at least in part, in *Ornithogalum*. Nucellar cells between the megagametophyte and the apex of the nucellus proper show signs of degeneration well in advance of any physical distortion of cell shape or of actual contact with the megagametophyte. Most notable of such signs are the condensation of their nuclei and the increased affinity for protein stains. Nucellar cells immediately surrounding
the megagametophyte exhibit some nuclear condensation and increased affinity for staining, but they also appear to become physically distorted. These centralmost cells of the nucellus, then, are probably genetically programmed to degenerate as are the previously mentioned cells, but because of their proximity to the megagametophyte, they experience the consequence of mechanical pressure as well. Cytoplasm of the nucellar cells which degenerate during megagametophyte expansion is most likely resorbed by the megagametophyte and used in its nutrition.

The nucellus in most plants probably has a significant function as a storage tissue for nutrients used by the megagametophyte, and possibly also in later stages by the embryo and endosperm. In Ornithogalum, the nucellus, except for the nucellar epidermis, accumulates large quantities of starch, but not of lipids. This is somewhat surprising in that lipids seem to be so important in other areas of the gynoecium in Ornithogalum. Lipids and starch are, however, the most prevalent storage materials generally found in nucelli (Tilton, 1974; Moskova, 1975; Pacini et al., 1975), but protein bodies may comprise a significant portion of the storage material in other cases (Horner and Arnott, 1965; Gori, 1976).

Although the nucellar epidermis in Ornithogalum does not proliferate to form a multiseriate nucellar cap as in some other members of the Liliaceae (Cave, 1955; Fulvio and Cave, 1964; Sterling, 1973b,c, 1975) and in many other taxa, it is quite a distinct structure. The large palisadelike cells with their proximally thickened walls and the very thick wall between the nucellar cap and the nucellus proper are characters similar to those seen in Ornithopus (Wojciechowska, 1972a), but more closely resemble those of Agave (Tilton, 1974). In both Agave and
Ornithogalum, the proximal end of the apicalmost cells of the cap has numerous vesicles which appear to be either fusing with or pinching off from the plasmalemma. The outer membrane of the vesicles is tri-partite and has an appearance similar to that of the plasmalemma. A detailed ultrastructural investigation is needed to determine if these vesicles merely represent wall synthesis or if they perhaps represent some sort of pinocytotic activity involved in the transport of materials synthesized by the synergid. This latter idea will be discussed more fully in a later section.

As in other species of Ornithogalum (Zabińska, 1972), the micropyle of O. caudatum is formed from the inner integument with the nucellar cap located at the proximal end. The micropyle is straight and does not curve or zig-zag as it does in some other Liliaceae, viz., Ornithopus (Wojciechowska, 1972a). Periclinal divisions in the micropylar region of the inner integument result in the integument being from three to five cells thick around the micropyle. This condition is also seen in other members of the lily family (Cave, 1955, 1974; Fulvio and Cave, 1964) as well as in other taxa, e.g., Pelargonium (Tsai et al., 1973).

Integumentary cells lining the micropyle in several taxa have been noted to be different from other cells. In Tricyrtis (Ikeda, 1902), they are not cuticularized and contain starch, while in several members of the Alsinoideae (Gibbs, 1907) and in Dodia and Richardsonia (Lloyd, 1899), they show differences in wall and cytoplasmic characteristics. In only two cases to date has any secretory activity been reported in association with the micropyle. Paspalum (Chao, 1971, 1977) has PAS positive material filling the micropyle, and Senecio aureus has a mucilaginous
substance in the micropyle which is thought by Mottier (1893) to be secreted by the synergids.

The secretory material in the micropyle of Ornithogalum stains positively for protein and RNA, but not for carbohydrate. On the basis of this histochemical analysis, the micropylar exudate in Ornithogalum has a much different consistency than does that reported by Chao (1971, 1977) for Paspalum which is all carbohydrate. The specimens used by Chao show fixation artifact, however, and so one should use caution when considering his data. The copious amount of exudate and the correlation in timing of its secretion with anthesis in Ornithogalum are suggestive of this material being involved in pollen tube growth and nutrition.

In Ornithogalum it appears as though most of this material is secreted by the nucellar cap with the inner integument contributing the balance. This unequal division of labor is suggested by the fact that a thick coating of the exudate is seen on the nucellar cap before any is seen on the inner integument and because the nucellar cap cells present a larger secretory face to the micropyle.

The most unique feature about the micropyle in Ornithogalum is the occurrence of a thin sheet of material across the exostomium. The sheet appears at about the same time the filiform apparatus develops in the synergids, but its true function can only be speculated upon at this point. It is possible that this hymen, if you will, forms a seal across the exostomium to prevent the general escape into the locule of a fluid substance, possibly of synergid origin, which causes directional growth of pollen tubes. It is also possible that the hymen is permeable enough to allow a small amount of the fluid to pass through it so that the hymen
becomes saturated. This small, localized concentration of a chemotropic substance at the exostome might then provide a sufficient stimulus to cause pollen tubes to turn and enter the micropyle.

Schnarf's (1929) definition of a hypostase as being lignified cells which appear in the early development of the ovule's chalazal end is not completely accurate for the condition in *Ornithogalum* and some other plants, e.g., *Agave* (Tilton, 1974). It is, however, superior to the definitions of Van Tieghem (1869) and Johansen (1928) who considered any chalazal modification to be a hypostase. The latter definition, then, would include such things as the podia described by Dahlgren (1940), the nucellar plasmodia, or pseudo embryo sacs described by Went (1910, 1926), and possibly also the pseudochalaza described by Goldfluss (1899) and Engell and Petersen (1976). A slight addition to Schnarf's definition might include the following.

**Hypostase**: A group of modified cells with lignified walls, generally within the chalazal region of an ovule, but which may surround a portion of the megagametophyte and extend part way into the micropylar half of the ovule. Lignification of the chalazal portion occurs at an early stage of ovule ontogeny but may continue into advanced stages in the micropylar portion. The function of the hypostase is not known with certainty, but it is thought to be involved in megagametophyte nutrition.

Carbohydrate and protein, along with the highest concentration of lipid in the ovule, accumulate in the hypostase of *Agave*. This coupled with the fact that the hypostase is located between the terminus of the vascular tissue and the megagametophyte led Tilton (1974) to postulate a nutritive role for the hypostase. The contents of some hypostase cells
in *Ornithogalum* stain intensely for carbohydrate, and at ovule maturity some starch may be found in the hypostase, but proteins and lipids do not accumulate. The hypostase in *Ornithogalum* thus does not appear to have a significant role as storage tissue like that of *Agave*. Considering, however, that it is positioned between the megagametophyte and the starch stores in the nucellus, and between the megagametophyte and the vascular tissue, it must be related in some way with the translocation of nutrients into the megagametophyte, and following fertilization, into the embryo sac.

According to Maheshwari (1950), some authors have suggested that the hypostase, and the epistase, act as barriers to prevent excessive expansion of the megagametophyte. This might be a plausible tenet if the nucellar cells which degenerate to accommodate megagametophyte expansion did so as a result of mechanical pressure, but as discussed previously, this degeneration seems to be genetically programmed so that the cells degenerate well in advance of physical contact with the megagametophyte.

The function, if any, of the one to three unique cells per hypostase, and of the intercalary bloc of four to six cells chalazad the hypostase proper remains a mystery.

Unlike some palms which may have as many as fifteen vascular bundles feeding one ovule (Uhl and Moore, 1971), *Ornithogalum*, like most ovules, is fed by only one vascular trace. The vascular tissue of *Ornithogalum* ovules was not studied in any great detail, but it appears to be amphicribral in nature, with phloem surrounding the xylem. In general, very little has been written about vascular tissue in ovules, but it seems
as though most do have amphicribral bundles. This is also the prevalent condition in stamens (N. R. Lersten, Iowa State University, personal communication, 1977). Other bundle types are found in ovules, however, as exemplified by *Chorzophora* (Bor and Kapil, 1976) in which the vascular bundles are reported to be collateral.

In addition to supplying vascular tissue to the ovules, the funiculus also seems to be responsible for causing the curvature which results in anatropous ovules. Curvature is produced by a greater number of mitotic divisions occurring on one side of the funiculus than on the other side. The funiculus may also contribute to the formation of an obturator as it does in *Tieghemopanax sambucifolius* (Rao, 1972) and in *Ornithogalum*.

Funiculi are not ubiquitous in their occurrence, however, as some members of the Palmae (Robertson, 1976) and of the Polygonaceae (Laubengayer, 1937) have ovules attached directly to the placenta without an interveining funiculus.

Unlike orchids, in which parietal cells never develop (Abe, 1972), *Ornithogalum gussonei* (Żabińska, 1972) and *O. caudatum* do form parietal cells. This feature seems to be a fairly common trait in the lily family (Schnarf, 1929; Wojciechowska, 1972a) and in other families as well (Schnarf, 1929). In both of the *Ornithogalum* species just mentioned, parietal cell activity results in two rows of either two or three parietal cells each. Cases of extreme parietal cell activity result in the formation of nucellar beaks as in *Euphorbia* (Lyon, 1898; Bor and Kapil, 1975), *Polygonum* (Engell, 1973), and *Persea* (Tomer and Gottreich, 1976). The lack of parietal cells in the Orchidaceae provides a good example of the tenet proposed by Coulter and Chamberlain (1909) that the evolutionary
trend is toward suppression of parietal cell activity and the eventual loss of parietal cells altogether.

According to Maheshwari (1950), the archesporial cell may either divide and give rise to a primary parietal cell and a primary sporogenous cell, or it may function directly as the MMC. In *Ornithogalum*, it is the latter case. The primary parietal cell and the archesporial cell are probably derivatives of the same mother cell. The division which gives rise to these two cells must be one of the earliest divisions to occur during ovule initiation as archespore differentiation is one of the first events to take place during ovule ontogeny.

The cell wall around the archespore exhibits greater staining intensity than do other cell walls of the nucellus proper, and, by the time meiosis occurs, the distinction of this wall is quite evident. In *Ornithogalum*, however, callose is apparently not deposited on this wall. This makes *O. caudatum* an exception to Rodkiewicz's Rule (1968, 1970) which states that species with monosporic type megagametophyte development deposit callose around the megasporeocyte antecedent to and during meiosis. The general absence of callose in the tetrasporic type megagametophyte development (Rodkiewicz, 1968, 1970) and its apparent nonoccurrence in *Ornithogalum*, however, indicate that its deposition is not a necessary prerequisite for megaspore formation.

Cytoplasmic inclusions in the MMC of the types described in some other members of the Liliaceae by some of the earlier authors on megasporogenesis, e.g., Sargant (1896), Bambacioni and Giombini (1930), Cooper (1935), Romanov (1936), were not found in *Ornithogalum*. As noted by Flint and Johansen (1958), such inclusions were generally
reported to occur only in plants with *Fritillaria* type megagametophyte development, and *Ornithogalum* is of the *Polygonum* type.

Flint and Johansen (1958) classified these inclusions into three categories, the largest of which was described as consisting of radiations extending from the nucleus to the plasmalemma. They considered these radiations to be real, but they concluded that the other two categories were actually radiations but that they had become distorted due to the chromic acid used in the fixing solution. Rodkiewicz and Mikulska (1965) have shown ultrastructurally that the radiations are parallel cisternae of ER. Radiations and other types of inclusions have not been reported to occur in the MMCs of other plant families.

As in most ovules (Maheshwari, 1941), meiosis of the megasporocyte in *Ornithogalum* results in a linear tetrad of megaspores, the chalazalmost of which becomes the functional one and gives rise to the megagametophyte. The first meiotic division produces two megaspores of unequal size, the micropylar megaspore being smaller than the chalazal one. In the second division, the micropylar megaspore produces two daughter cells of equal size, but the chalazal megaspore divides unequally with the chalazalmost daughter cell being the larger of the two. The staining reaction of the nuclei in the three micropylar megaspores is very intense and is similar to the staining reaction of the nuclei in the nucellar cells which degenerate in advance of the expanding megagametophyte. In both cases, the nuclei seem to condense antecedent to cellular degeneration, and it may be that condensation of the nucleoplasm is responsible for the increased affinity for stain. It is also possible that the increased staining reaction reflects a peak of activity in the
nucleus as it directs preparations for autolysis.

Two ideas have been proposed as to why the chalazal megaspore is usually the one which develops into the megagametophyte. Dixon (1936) suggested that a mitosis-inducing hormone is synthesized in the chalaza, and Koernicke (1901) and Erdelská (1975b) suggested that its greater proximity to the vascular tissue is the reason. Interestingly, Dixon's suggestion came before the discovery of IAA, but in any event, it does not seem to be plausible. If a mitosis-inducing hormone were produced in the chalaza, one would expect that within such a confined system as an ovule that a very localized effect, viz., one cell, as required by Dixon's hypothesis would be nigh impossible. The latter idea of proximity to vascular tissue is, however, a bit more tenable, but it also suffers from being subject to the same criticisms that the hormone hypothesis is. In addition, neither hypothesis proffers an explanation for bisporic, tetrasporic, or Oenothera type developmental systems.

That meiotic divisions produce megaspores of equal size in tetrasporic systems and megaspores of unequal size in monosporic systems suggests that some sort of polarity is already present and genetically programmed in the monosporic system before the divisions occur. Environment would provide a secondary factor, with the absence of callose allowing a division stimulant from the nucellus to pass into the megaspore. The lack of callose in tetrasporic systems and the selective absence of callose over the apex of the cell which becomes the FM in both the Polygonum and Oenothera types of monosporic systems support this hypothesis. Just what the stimulant is, however, is certainly a matter of conjecture, as it could be a hormone or a nutrient(s) or both.
There are patches which are devoid of callose in the meiotic cross-walls of Epilobium (Rodkiewicz, 1973) and of Fuchsia (Rodkiewicz and Kadej, 1974) which would allow free movement of materials between the megaspores as the authors suggested. The cell which receives the stimulant directly from the nucellus will receive the greatest share and would thus most likely become the FM just as if callose were uniformly deposited across the meiotic cross-walls. This factor, then, also allows Epilobium and Fuchsia to fit the hypothesis just presented. The situation with Ornithogalum, however, is a mystery unless callose is present but was not detected. Tests with fresh material prepared by squashing are needed to confirm or to disprove the results obtained with resin-embedded specimens.

Cases of more than one megaspore being functional have been reported for other species of Ornithogalum, viz., O. umbellatum (Desole, 1947; Czapik, 1966) and O. gussonii (Żabińska, 1972), as well as in many other taxa (Schnarf, 1929). Examples of this were not detected in the more than one thousand O. caudatum ovules observed, nor were any structures seen which resemble haustoria as reported for the FMs in Tricyrtis (Ikeda, 1902), Sedum and Rosularia (Mauritzon, 1933), and in Galium (Fagerlind, 1937).

During meiosis in two ferns, Polypodium aureum (Marengo and Marengo, 1972) and Onoclea sensibilis (Marengo, 1977), anaphase II is characterized by a plate of mitochondria forming across the MMC. Amyloplasts are found here also, but not in as great a concentration and lipid bodies are distributed randomly. No such organelle distributions have been reported in angiosperms, but a definite arrangement of organelles has
been reported to occur during mitotic divisions of the FM in two cases. In *Epilobium* (Rodkiewicz and Bednara, 1974), amyloplasts are polarized at both poles of the FM and the dictyosomes are perinuclear while, in *Ornithogalum gussonei* (Zabińska, 1972), there are two polar vacuoles with the nucleus in between. As in *O. gussonei*, FMs of *O. caudatum* have two polar vacuoles with a centrally located nucleus, but a few FMs were seen with only one vacuole. In all specimens of *O. caudatum*, however, a very definite perinuclear distribution of organelles was encountered. This perinuclear distribution also occurs during all subsequent mitotic divisions of the megagametogenesis process.

The perinuclear distribution, or nuclear halo, appears to be equal around the FM nucleus preceding the first mitotic division of the megaspore, and it also appears to be equal around the primary chalazal and primary micropylar nuclei preceding the second division. The equal distribution around the nuclei is probably a mechanism to insure each daughter cell of receiving an equal share. In the third division, there is an equal distribution between the chalazal pair, but an unequal distribution between the two micropylar nuclei.

The micropylarmost nucleus of the micropylar pair has more organelles around it, particularly on its micropylar side. This nucleus gives rise to the two synergids, and in that the synergids are highly metabolically active cells, it stands to reason that there is a mechanism to insure that they will be well-endowed from the outset. In contrast, the egg and polar nucleus, derived from the chalazal nucleus of the micropylar pair, have a passive role during fertilization and do not require as extensive an organelle complement as do the synergids.
If this premise correlating organelle distribution between nuclear halos antecedent to a division, and the relative metabolic activity of the daughter cells, is valid, it should be reflected in the chalazal set of nuclei as well as in the micropylar set. The chalazal quartet of nuclei produces the three antipodals and a polar nucleus, and each of these derivatives is relatively inactive when initially formed. They do not exhibit any pronounced metabolic activity until after fertilization has occurred. Thus, as predicted by the premise, no particular nucleus of the chalazal pair receives a greater share of organelles than does the other, and all four daughter nuclei are equally endowed.

As in *Crocus* where it was first described by Hofmeister (1857) and since then in many other plants, a strand of cytoplasm which contains the polar nuclei traverses the central cell's vacuole in *Ornithogalum*. The formation of this strand in *Ornithogalum* must be very rapid as no intermediate stages of its development were encountered. From available evidence, however, it appears that the strand initially contains only the micropylar polar nucleus, and that after the two nuclei approach one another, the chalazal nucleus becomes included within it. According to the maxim postulated by Ikeda (1902), such strands provide a means by which the polar nuclei can unite, and by which the antipodals and egg apparatus can subsequently maintain communication. It also seems very likely that this strand provides a direct avenue by which the sperm affecting double fertilization may travel to reach the fusion nucleus.

This question is definitely one area of the reproductive process in angiosperms that needs to be investigated in greater detail. The mechanism by which polar nuclei migrate within the central cell is not known,
nor is it known how sperm move from the point of their discharge in the synergids, let alone the mechanism by which they are attracted to the appropriate female nuclei.

The central cell seems to serve as a repository for storage materials to be used in embryo and endosperm nutrition following fertilization. It quite possibly also serves as a nurse cell to the egg apparatus. The accumulation of fats and carbohydrates in the central cell has been noted to occur in a long list of plants, and in some taxa the central cell may become completely engarged with storage products. In addition to the central cell storing carbohydrates and fats, Ryczkowski (1964) and List and Steward (1965) have shown that it may accumulate amino acids and inorganic salts in the vacuole.

Some ovules have become variously modified to enhance the large influx of material into the central cell. Some species have developed Wandlabrinthes in either or both the chalazal and micropylar end of the central cell. Other taxa have developed integumentary tapeta around the central cell, and yet others have antipodals which develop Wandlabrinthes, proliferate in number, or are otherwise modified to introduce a large amount of material into the central cell. As an extreme example of modifications to acquire storage materials, the central cell of Aquilegia vulgaris produces haustoria which invade the chalaza (Rifot, 1973).

Antipodals historically have been one of many enigmatic features of the angiosperm megagametophyte. But, the more recent papers concerning their anatomy seem to indicate a growing trend among most contemporary investigators toward considering them as vehicles for the transport of nutrients into the megagametophyte and embryo sac in ovules where the
antipodals persist. This school is based on the hypothesis proposed by Westermaier (1892), which contrasts that tendered by Vesque (1879) which states that antipodals are evolutionary curiosities of no functional significance.

Evidence from Ornithogalum tends to corroborate Westermaier's school. Antipodals in O. caudatum are initially vacuolate but gradually fill with cytoplasm. By anthesis they are completely filled with dense cytoplasm and storage products, viz., starch. The timing of these events indicates that the antipodals in O. caudatum probably are more functional in embryo sac nutrition than in megagametophyte nutrition. Passage of materials through the antipodals during megagametophyte development is not to be ruled out, however. In all probability, a goodly portion of the total influx is through these cells with the balance passing through the lateral walls of the central cell. In Ornithogalum as in most angiosperm ovules, little, if any, nutrient materials enter the megagametophyte or embryo sac through the synergids.

Antipodals of many species have from one to several modifications indicative of their role in megagametophyte and embryo nutrition such as being cells with high metabolic activity and/or being cells with anatomical features for material transport. Specific characters include: substantial amounts of dense cytoplasm with abundant mitochondria, ribosomes, and RER; the presence of protein, lipid, or starch as storage products; the formation of Wendlabinthes and haustoria; high levels of RNA; multiple number of nuclei--up to twelve per antipodal; endopolyploid nuclei of up to 48 and 64 n; and cell proliferation of up to as many as several hundred antipodals per ovule.
The multinucleate condition probably represents a multicellular condition where the system either has not advanced to the stage of forming cell walls or where cell wall formation has been suppressed. Provided that the sphere of influence exerted by any given nucleus does not interfere or compete with that of another nucleus, the multinucleate system would seem to be energetically more economical. The multicellular condition is more prevalent, however, implying that it has other qualities which are of greater significance than the energetics of wall formation. Perhaps there is a detrimental effect from an overlapping of spheres of influence as nuclei approximate one another as a consequence of cyclosis.

As noted previously, those plants which have ephemeral or nonfunctional antipodals seem to have evolved some other mechanism for introducing large amounts of material into the megagametophyte. The development of Wandlabrinthes in the central cell and/or the development of integumentary tapeta are the principal such mechanisms. New research and a survey of extant literature to correlate the incidence of ephemeral and persistent antipodals with the presence or absence of either Wandlabrinthes in the central cell or of integumentary tapeta would be of value in substantiating the antipodals' role in megagametophyte and embryo sac nutrition. Research is also needed to corroborate the various implications, viz., Westermaler (1890), Brink and Cooper (1944), and Maze and Lin (1975), which involve antipodals in endosperm development.

Typical of the female gamete in angiosperms, the egg in *Ornithogalum* is smaller than the synergids, has a large micropylar vacuole, and its nucleus is located in the chalazal end with most of the cytoplasm. This
polarity of the egg, and particularly the chalazal location of the nucleus, is possibly an early expression of the asymmetric division of the zygote. It is also possible that there is some sort of polarity within the entire megagametophyte that is related to the chalazal movement of sperms after they are released into the synergids. The mechanism by which sperm reach their mate nuclei is unknown, but if it is any sort of chemical gradient, or perhaps an electrical field, it would seem most likely that both sperms would have a similar response, i.e., both would respond positively or both would respond negatively. A micropylar position for the egg nucleus would not allow both sperms to move in the same direction.

Another common feature of the angiosperm egg, also found in *Ornithogalum*, was first described in *Hepatica* by Mottier (1895). This feature is the chalazal attenuation of the cell wall from its thickest point between the egg and synergids to the point where no wall is formed around the chalazal pole of the egg. The central cell commonly does not form a wall here either, so these two cells usually are separated only by their respective plasmalemmas. There are exceptions, however, viz., *Bellis* (Engell and Petersen, 1977), in which the egg is completely surrounded by cell wall.

The female gamete has a passive role in the fertilization process, and so it is not surprising that the egg's organelle content reflects this passive existence. One does not encounter an abundance of mitochondria, ribosomes, ER, plastids, or dictyosomes. Eggs may contain relatively small amounts of starch or lipid as storage products, and this seems to be a more frequent condition than the apparently rarer
condition of being devoid of such materials as is *Ornithogalum*. The organelles present probably represent the modicum required to maintain basal metabolism until fertilization occurs. After fertilization, there is commonly a general increase in the incidence and apparent activity of all organelles in the zygote over that seen in the egg.

The synergids of *Ornithogalum* are apparently unique in having a cell wall which completely invests them and which appears to have no regions where discontinuities exist. In all cases reported to date, cell wall material is either completely lacking over the chalazal end of the synergids or it occurs to some degree, but it previously never has been reported to be entire. The synergid walls in *Ornithogalum* do thin chalazally, however, and are thinnest adjacent to the egg. In this respect, *Ornithogalum* is similar to all other reports for angiosperms. These features of chalazal attenuation and either a complete or a partial lack of a cell wall over the chalazal end of the synergids has been related to the movement of sperm out of the synergids, a topic to be considered later.

Synergids are undoubtedly the most metabolically active cells of the megagametophyte, and probably of the entire ovule. They typically have dense cytoplasm and are very well-endowed with ER, ribosomes, dictyosomes, and mitochondria. Histochemical tests for nucleic acids and proteins indicate substantial amounts of these compounds in the synergids of *Ornithogalum* and other taxa as well. Their nuclei are typically large, they may be lobed, and, as in *Ornithogalum*, each nucleus may have one very large nucleolus with a large pars morpha phase. The incidence of plastids may range from none to many while microbodies are always few in
number. A build-up of large amounts of starch or lipid as storage products in synergids is known to occur, but synergids more commonly have only a modicum of storage materials or none at all. With their characteristic filiform apparatus, synergids thus appear to be cells which have been modified to become extremely specialized secretory cells, and their role in the reproductive process will be considered shortly.

The presence of a filiform apparatus is the character which immediately identifies a cell as a synergid, except in *Plumbago* (Cass, 1972; Cass and Karas, 1974) where there are no synergids and the filiform apparatus occurs in the egg. This structure is the single largest localized expanse of cell wall material in the Plant Kingdom, and is indeed a Wordlabrinthe in the most literal sense of the word. The filiform apparatus in *Ornithogalum* is quite extensive, and, similar to *Torenia* (Pluijm, 1964), the numerous vesicles and small vacuoles which surround it suggest that it serves as the pathway across which secretory products synthesized by the synergids can exit from the megagametophyte.

Some Brief Comments on Events Preceding Fertilization

In considering the gynoecium as a reproductive system, one should realize that all of the various parts must function properly at the correct time and in the proper sequence. The whole purpose of the system is to affect double fertilization and to allow the new sporophyte generation to develop to a point where it can be self-sufficient when separated from the mother tissues. The major events and processes which lead up to syngamy and triple fusion will be dealt with in the order in which they occur, and they include: pollen recognition and germination, floral
responses to pollination, pollen tube growth, and pollen tube entry into the megagametophyte.

As is often done in general botany laboratories, one may place pollen grains in a ten percent sucrose solution at room temperature and obtain a vigorous culture of growing pollen tubes in a short time. Not all types of pollen will do this, but most do. This would seem to indicate that all pollen requires for germination is moisture, an energy source, and a moderate temperature. Yet, when pollen from one species lands on the stigma of another species, the grains often do not germinate, particularly if the two plants are taxonomically distant. This would seem to indicate that some recognition mechanism exists between stigma and pollen which either enhances or inhibits pollen germination.

Stanley and Linskens (1965) and Mäkinen and Brewbaker (1967) have postulated that enzymes diffuse from pollen into the exudate where they release free sugars from phenolic glycosides to provide proper osmotic conditions for pollen germination. Konar and Linskens (1966a) speculated that fatty acid esters from stylar exudate function as a liquid cuticle around pollen to prevent water loss during germination; the water for pollen germination coming from the stigma also. This may be fine for water relations with wet stigmas, but what about dry stigmas?

Mattsson et al. (1974) described an extracuticular layer of proteinaceous material which they termed the pellicle, and concluded that it is the pollen-trapping and -hydrating agent for dry stigmas. A goodly number of investigations with wet stigmas have given support to the just mentioned ideas concerning their water relations, but dry stigmas have been generally overlooked, and, to date, the report of Mattsson et al.
(1974) is the only one of its kind. More information is obviously needed in this area if mankind is to one day learn how to overcome this barrier in affecting interspecific crosses where pollen germination is a major factor.

The phenolic compounds within the stigmatic exudate have been proposed to serve as a nutrient source during the germination and initial growth phases of pollen grains (Martin, 1969, 1970a,b) as have the sugar moieties of the phenolic glycosides (Stanley and Linskens, 1965; Mäkinen and Brewbaker, 1967). The fat moiety of the stigmal exudate, at least in Petunia (Konar and Linskens, 1966a), seems not to be involved in pollen grain nutrition. In general, stigmatic exudate seems to serve only in a minor capacity as a nutrient source for pollen tubes, but this is not surprising considering the following four facts: (1) pollen grains become engorged with stored nutrients during their development; (2) pollen tubes produce periodically spaced callose plugs behind the growing tip; (3) stigmatic tissue is only a few cell layers deep at the most; and (4) stylar exudate has been shown to be an important nutrient source for growing pollen tubes.

Martin (1969, 1970a,b) has proposed that the phenolics of the stigmatic exudate are involved with the recognition reaction between pollen and stigma, as well as with the inhibition of germination of fungal spores. The inhibition/stimulation reaction seems to revolve around the interaction of the phenolic compounds with indole-acetic-acid-oxidase (Van Sumere, 1960; Martin and Ruberté, 1972). Recognizing and enhancing the germination of like pollen and, conversely, inhibiting the germination of unlike pollen are probably the most significant functions of the stigma.
Of the myriad of modifications to the basic plan of a flower, I find sensitive stigmas to be one of the most fascinating. This fascination is in part a curiosity which stems from their occurrence in only some thirty genera of five families (Newcombe, 1922, 1924). They are also interesting from a scientific standpoint in that they respond to both mechanical and chemical stimuli, but the responses are different. Stigmas will reopen following mechanical stimulation, but not after being pollinated.

The ability to distinguish between pollination and incidental irritation prevents the ovary from undergoing its physiological post-pollination responses unnecessarily (Sinyukhin and Britikov, 1967). One species, *Spathodea campanulata* (Newcombe, 1924) requires both mechanical and chemical stimulation to close the stigma. This necessity for both types of stimulation may represent a mechanism to conserve energy as in the other species, and, in addition, a mechanism to prevent fertilizations that might result from pollinations by a promiscuous vector carrying foreign pollen. Quite probably, requirements for the proper mechanical stimulus are met by the specific size and/or weight characters of the actual vector, but not by other potential vectors found in the range of *Spathodea*.

As just alluded to, flowers exhibit physiological responses to pollination. Some responses may be induced by the mere presence of pollen on the stigma while others are induced only after pollen tubes have actually started growing down the style. Working with *Zea, Lilium, Incarvillea*, and several other flowers, Sinyukhin and Britikov (1967) demonstrated that chemical interchanges between pollen and stigma generate electrochemical action potentials which travel down the style and
initiate physiological responses in the ovary. The first such response is an increase in the respiration rate.

In contrast, Gilissen (1977) found that wilting of the corolla in *Petunia* is initiated only after pollen tubes penetrate the stigmatic surface, and that the wilting reaction is controlled by the style. Wilting in *Petunia* begins as soon as the stigma is penetrated (Gilissen, 1977), but, in *Ornithogalum*, this reaction seems to require about twelve hours to become noticeable, and it takes four to five days for the tepals, stamens, and style to be completely shriveled and appear dry. Gilissen (1977) concluded that the wilting response is a signal by the flower to the environment that the corolla is no longer functioning due to pollination. It seems more likely, however, that wilting is a result of the effluence of cytoplasm from the corolla and style into the ovary, and that any signaling to the environment is only of secondary consequence.

The style is the main sink for organic materials from other floral parts immediately after pollination (Linskens, 1973, 1974a,c), and, although not proven experimentally, it seems likely that this influx is correlated with stylar hormone synthesis. The style has been shown to exhibit increased IAA production in a matter of hours after pollination (Lund, 1956), and this high IAA level has been shown to cause a subsequent increase in gynoecial ethylene production (Hall and Forsyth, 1967; Forsyth and Hall, 1969; Lipe and Morgan, 1973). In turn, high ethylene levels cause an increase in the sucrose/reducing sugar ratio in other floral parts (Nichols and Ho, 1975; Nichols, 1976), and the ovary supersedes the style as the main sink within a short while after pollination (Linskens, 1973, 1974a,c). The influx of sugars and inorganic substances
from other parts of the flower into the ovary has been demonstrated by several investigators, viz., Tupý (1961), Arditti (1969), Arditti and Flick (1976), Linskens (1973, 1974a,c), Nichols and Ho (1975), and Nichols (1976).

From the foregoing information, then, one can easily deduce the following as being the early sequence of events in the gynoecium and other floral parts: (1) secretory activity by the stigma prepares the stigma for receptivity; (2) pollen lands on the stigma; (3) enzymes diffuse from the pollen into the stigmatic exudate; (4) recognition factors correspond; (5) pollen grains germinate and grow into the style; (6) an electrochemical action potential travels down the style; (7) the ovarian respiration rate increases; (8) the style becomes a temporary sink; (9) IAA production is stimulated; (10) ethylene production is stimulated; (11) organic and inorganic substances from all nonovarian floral tissues are mobilized; (12) the ovary becomes the main sink; (13) nonovarian floral parts wilt as their cytoplasm moves into the ovary.

Before styles wilt, however, pollen tube growth through the stylar portion of the gynoecium must be completed. In serving as a passageway for pollen tubes to grow through from stigma and ovary, styles function as a screening agent for undesirable pollen (Whitehouse, 1950), as the site for most incompatibility reactions to occur (Linskens, 1975; Heslop-Harrison, 1975), and as a source of nutrition for growing pollen tubes (Tupý, 1961; Kumar and Hecht, 1970; Kroh et al., 1970, 1971; Kroh, 1973; Kroh and Helsper, 1974; Rosen, 1971; Rosenfield and Loewus, 1975; Mascarenhas, 1975; Campbell and Ascher, 1975; Wolff, 1975). These four functions are, of course, in addition to those just mentioned concerning
control of early post-pollination hormonal activity in the gynoecium.

It should be noted that, although not yet experimentally proven, Muir (1942) speculated that growing pollen tubes secrete enzymes which activate growth hormones found in inactive forms within the stylar exudate. If this is so, it would mean that stylar control of hormonal activity in the gynoecium is actually a consequence of pollen-stylar exudate interaction and not a direct function of the style per se as proposed by Gilissen (1976, 1977).

Although most authors (see previous listing) have indicated that stylar exudate is primarily a source of polysaccharides for pollen tubes, Campbell and Ascher (1975) have shown it to be a source of nucleic acid precursors, Wolff (1975) of amino acids for both protein synthesis and as a nitrogen source, and Pfahler and Linskens (1974) have implicated that stylar exudate may also provide various minerals. Lipids are noticeably lacking from this list which seems to indicate that the large quantity of lipoidal material in the exudate of Ornithogalum is unique. Considering the high incidence of lipid in other parts of the gynoecium and its apparent overall importance in the metabolism of Ornithogalum, however, its occurrence in the stylar exudate is not surprising.

Spherosomes are known to be a source of lipid (Gahan, 1968), and the spherosomes, in conjunction with the microbodies (Vigil, 1973; Hanzely and Vigil, 1975) located in the transmitting tissue are probably a source of some of the lipid in the exudate. The other lipid material in the exudate is undoubtedly derived from the plastid-ER association found in the transmitting tissue as discussed previously. Vasil'ev (1970) has ascribed most of the secretory activity of the transmitting tissue
in *Lilium* to dictyosomes, but in *Ornithogalum*, dictyosome activity does not seem to be as prevalent. Ribosomes, ER, plastids, spherosomes, and microbodies, in addition to dictyosomes, are all involved in synthesizing stylar exudate in *Ornithogalum*, and without a quantitative analysis of the exudate it is impossible to say which moiety is most prevalent.

Although the actual growth of pollen tubes was not monitored in *Ornithogalum*, the ex post facto evidence indicates that they have an ectotropic growth habit along the entire length of the transmitting tissue. This is the usual case for hollow styled flowers, while in solid styled flowers an endotropic habit is the rule (Vasil and Johri, 1964). Once *Ornithogalum* pollen tubes reach the nucellar cap, however, their mode of growth becomes endotropic and they grow between the nucellar cap cells. To do this, the pollen tubes must be able to synthesize pectinases as do the pollen tubes of solid styled species (Pluijm and Linskens, 1966; Jensen and Fisher, 1969). Also, considering that most of the polysaccharide moiety of stylar exudate is pectin (Rosen and Thomas, 1970; Kroh, 1973; Sassen, 1974), pectinase synthesis by *Ornithogalum* pollen tubes must also be of considerable importance in regard to their obtaining carbohydrate for growth. The synthesis of cellulase is probably of consequence only when a tube reaches the filiform apparatus.

One of the perpetual enigmas concerning pollen tube growth is the mechanism by which they are guided, if indeed they are guided, to the megagametophyte. Brink (1924) noted three possibilities; (1) mechanical guidance by anatomical features of the gynoeclium, (2) chemotropic guidance by secretions of the gynoeclium, and (3) pure chance. A combination
of mechanical and chemical influences as a fourth alternative is also possible. Just to be complete, however, all remaining combinations of the three choices might be considered, but the four that are enumerated seem to be the most reasonable. After having studied the system in *Ornithogalum* and after having considered all the extensive evidence and the theories presented in the literature review, it is my personal belief that in all likelihood the direction of pollen tube growth is influenced by both mechanical and chemical factors.

The extent of one factor's influence relative to another factor is probably a consequence of the gynoecial anatomy of the flower under consideration. Plants with solid styles and/or elaborate obturators would seem likely to exert a greater degree of mechanical influence than do those plants with hollow styles and/or no obturators. In the latter type of plants, the direction of pollen tube growth is probably more dependent upon chemical stimuli than is directional growth in the former plant type.

Historically, the influence of ovules on the final portion of a pollen tube's growth has been a point of major interest among botanists studying pollen tube growth and sexual reproduction. Influence by an ovule seems to emanate from the synergids, and some ovules, including *Ornithogalum*, seem to have a second center of influence, viz., the micropyle. Miyoshi (1894), who believed that pollen tubes grow mechanically from stigma to locule, observed what he termed secretory drops at the micropylar exostome and concluded that the drops exert a chemotropic effect on pollen tubes such that the tubes are attracted to the micropyle. The hymen found in *Ornithogalum* might well serve as both a barrier
to prevent a flooding of the locale with micropylar exudate and as a repository surface for a localized concentration of the secretory material. This localized concentration might then provide the stimulus sufficient to cause a pollen tube to turn and enter the micropyle in a manner analogous to that suggested by Miyoshi (1894).

That the synergids are involved in directing pollen tube growth was perhaps first suggested by Schacht (1857) who named the filiform apparatus and proposed that the striations he observed in it serve as mechanical guides. Lloyd (1899) opined that the synergids are both the source and the center for distribution of chemotropic substances in the ovary, and Ishikawa (1918) was even more specific than Lloyd. He stated that the filiform apparatus and the secretory product of the synergids are responsible for chemotropically guiding pollen tubes. Based on these early ideas and on contemporary ultrastructural evidence, viz., Pluijm (1964); Van Went and Linskens (1967), Van Went (1970a,c), Schulz and Jensen (1968a), Jensen and Fisher (1968), Cocucci and Jensen (1969b), and Mogensen (1972), it is now almost universally agreed that synergids produce a substance capable of influencing pollen tube growth, and that the filiform apparatus is the specific target which pollen tubes grow toward.
SUMMARY

Gynoecial and ovule development in *Ornithogalum caudatum* Ait. were investigated using various techniques of LM, SEM, and TEM. LM histochemistry was conducted to determine the presence or absence of carbohydrate, protein, lipid, and nucleic acids. With the noted exceptions of starch, callose, RNA, and DNA, no further specific compound identifications were made. Gynoecia were studied from the time of carpel primordia initiation up through carpel dehiscence, including the dehisced carpels. Ovules were studied from the time of their initiation up to fertilization, but a few post-fertilization observations were made on the micropyle. Some observations were also made on pollen tubes in the style and in the ovary, but pollen tube growth per se was not monitored.

An extensive literature review was made which covers some historical information, gynoecial morphology and anatomy, and sexual reproduction in angiosperms from the female aspect. The historical notes and the material on floral morphology were included as matters of intellectual interest, and also to develop a more comprehensive understanding of what a gynoecium is structurally and functionally.

Carpels

A basal meristem initiates dome-shaped primordia. Once initiated, primordia grow primarily by cell expansion rather than by cell division, but occasional mitotic figures are seen throughout carpel growth. The centripetal infolding of carpels and the acropetal fusion of the abaxial surfaces of adjacent carpels form the ovarian locules and the intervening septa. Radial fusion is not complete, and this results in the formation
of one long but narrow chamber in each septum. The epidermal cells lining the lumen of each chamber become secretory and produce nectar. The nectar is exuded from the upper end of these septal nectaries at the time the stigma becomes receptive.

During meiosis, a unique type of idioblast differentiates in the cortex of the carpels. Beginning near the base of the ovary, these idioblasts develop in close association with the lateral veins. By anthesis they are found throughout the carpels, but are most highly concentrated in the region around the septal nectaries and the lateral veins. The texture and morphology of the idioblastic material changes following anthesis, and the material eventually disappears. The contents of these cells, which stain for protein and carbohydrate, may represent a storage form of some substance utilized by the septal nectaries.

Crystalliferous idioblasts differentiate at an early stage of carpel development. They are found as individual cells or in clusters of several cells and are oriented periclinally. Although not followed ontogenetically, ex post facto evidence indicates that some crystalliferous idioblasts may grow intrusively into one or more neighboring cells. Numerous crystals form together as a fascicle within a matrix of protein and carbohydrate material. The individual, hexagonal raphide crystals develop within a hexagonal sheath formed by lamellae. The lamellae consist of minute spheres and each lamella is separated from the next by a series of tubules. Lamellae may anastomose with other lamellae or they may terminate abruptly. The crystals are later catabolized, as are eventually the lamellae and the matrix material.
The principal carpellary vasculature consists of one dorsal vein, two ventral veins, and two lateral veins. A reticulum of minor veins interconnects the major veins, but most of these smaller veins do not develop until after anthesis. A very distinct type of cell develops in conjunction with the minor veins. When first formed, these cells are densely cytoplasmic but they later become vacuolate. These unique cells are thought to function in the loading of the minor veins with carpellary photosynthate.

Stomates and a multi-layered chlorenchyma develop post-anthesis. Also following anthesis, lipotubuloids form in the outer epidermis, but they eventually become catabolized. The lipotubuloid cycle probably represents a mechanism for storing excess carpellary photosynthate for later use by the developing seeds. Dehiscence of the carpels occurs due to inrolling of the greatly thickened outer wall of the inner epidermis in a manner similar to fern annuli.

Stigma and Style

Epidermal cells at the apex of each carpel become distinguishable as stigmatic papillae shortly after stylar elongation begins. Papillae have rugose walls, and by about twenty-four hours after anthesis papillae have secreted a small amount of material onto their surface. Stigmatic secretion is coincident with that of the septal nectaries and denotes that the gynoecium is physiologically receptive for pollination.

Stylar elongation begins at an early stage of gynoecial development but does not terminate until after the final burst following anthesis. The style is vasculated by a continuation of the dorsal veins from
the subtending carpels. The hollow stylar canal becomes filled with a carbohydrate, protein, and lipoidal exudate just prior to anthesis. The exudate is synthesized and secreted by the stylar transmitting tissue in three phases. Each phase is characterized by a specific organelle population in the transmitting tissue. In the first phase, the cells are highly vacuolate and RER and ribosomes predominate, and, in the second phase, starch-containing plastids with a very dense stroma are ensheathed by SER. The third phase is distinguished by numerous dictyosomes and large transfer areas in the anticlinal walls. Styles and perianths are withered and dry in appearance four to five days after anthesis. Cytoplasm from these organs is apparently mobilized and translocated into the ovary for use by the fruit and developing seeds.

Ovules

The ovules are anatropous and crassimucellate with up to six parietal cells. Ovules are initiated acropetally along the lateral adaxial margins of the carpels. Inner integument initiation is concurrent with archespore differentiation, while outer integument initiation and funiculus elongation are prevenient to meiosis. During meiosis, the placenta and the base of each funiculus proliferate to form an obturator. The obturator is the ovarian portion of the transmitting tissue and, as such, is a secretory tissue. The nucellar cap also becomes very distinct as meiosis progresses. The micropyle is formed by the inner integument and it fills with an exudate secreted by the nucellar cap and the inner integument. A membranous sheet covers the exostome and is thought to serve as a repository surface for a localized concentration of a
chemotropic substance to influence pollen tube growth.

Meiosis results in a linear tetrad of megaspores, the chalazalmost of which becomes functional. Mitosis of the functional megaspore nucleus ensues, and the resulting process of megagametogenesis follows the Polygonum type. During megagametogenesis, organelles within the developing megagametophyte form perinuclear halos antecedent to a mitotic division. It is thought that the differential distribution of organelles preventing the third division represents the relative metabolic activity of the daughter cells. This follows from the fact that the synergid mother nucleus has the most organelles in its halo.

The three antipodals are initially vacuolate, but gradually become densely cytoplasmic and by anthesis they have accumulated a store of starch. Antipodals are proposed to serve as victualating agents for the megagametophyte. A cytoplasmic strand, within which the polar nuclei fuse, traverses the central cell. This strand is thought to serve as a communication link between the antipodals and the egg apparatus, and as a means for one sperm to reach the fusion nucleus.

The egg apparatus consists of one egg and two synergids. A large vacuole is located in the micropylar end of the egg while the nucleus and most of the cytoplasm are in the chalazal end. This polarity is probably reflective of the unequal cleavage division of the zygote. The synergids are highly metabolic cells well endowed with abundant ER, ribosomes, dictyosomes, and mitochondria. They are thought to secrete an agent which controls the final part of pollen tube growth. The most characteristic feature of synergids is the filiform apparatus, a massive proliferation
of their micropylar wall. The filiform apparatus serves as a transfer wall for the agent synthesized by the synergids, and, because of its size, it probably has the highest concentration of this substance in the ovule. The filiform apparatus is thus proposed to be the target toward which pollen tubes grow.
LITERATURE CITED


Brink, R. A. 1924. The physiology of pollen. IV. Chemotropism; effects on growth of grouping grains; formation and function of callose plugs; summary and conclusions. Amer. J. Bot. 11:417-436.


Hanf, M. 1935. Vergleichende und entwicklungsgeschichtliche Unter-


Kwiatkowska, M. 1972b. Changes in the diameter of microtubules connected with the autonomous rotary motion of the lipotubuloids (elaioplasts). Protoplasma 75:345-357.


Matile, Ph. 1968. Aleurone vacuoles as lysosomes. Z. Pflanzenphysiol. 58:365-368.
Matile, Ph. 1975. The lytic compartment of plant cells. Springer Verlag, New York.


Satina, S. 1944. Periclinal chimeras in Datura in relation to development and structure (A) of the style and stigma (B) of calyx and corolla. Amer. J. Bot. 31:493-502.


Tkachenko, G. V. 1959. The part played by stigma secretions in the pollination of the grapevine *Vitis vinifera*. Bot. Zh. 44:963-967. (Engl. summ.)


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## APPENDIX A. KEY TO LABELING

<table>
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APPENDIX B. FIGURES
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25. X 78,700
26. X 46,700
27. X 18,000
28. X 42,000
29. X 26,000
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65. X 123,500
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88. X 13,500
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89. X 40,700
90. X 37,500
91. X 24,400
92. X 32,650
Fig. 93. Portion of chloroplast from recently opened carpel of flower bud illustrating intensely osmiophilic lipid body inclusions. X 63,700

Figs. 94-96. Chloroplasts from old carpels in early stage of cytoplasmic degeneration prior to carpel dehiscence showing digestion of lipoidal inclusion bodies

94. X 43,200
95. X 13,700
96. X 16,800
Fig. 97. Cytoplasm from carpel cortex between two chloroplasts containing numerous ER vesicles. X 37,600

Fig. 98. Starch-containing chloroplast from post-anthesis carpel in close association with microbody and dictyosome. X 29,000

Figs. 99-100. Chloroplasts from older carpels illustrating large grana stacks typical of older carpels

99. X 19,200

100. X 20,700
Figs. 101-105. Carpellary idioblasts

101. Trans-section of gynoecium illustrating three crystaliferous idioblasts and numerous cells of an idioblastic type unique to Ornithogalum (lower portion of micrograph). X 154

102. Two crystalliferous idioblasts with crystals occurring in fascicles of orderly files. Dark material around crystals is matrix in which crystals form. Matrix stains positively for protein and carbohydrate. Basic fuchsin and napthol black. X 341

103. Raphide fascicle protruding from mother cell. Crystals parallel long axis of gynoecium. X 817

104. Trans-section of two crystalliferous idioblasts. Note more random organization of crystals in these cells, particularly cell on right, as compared to Figs. 102 and 105. Hexagonal shape of some crystals denoted by arrows. X 374

105. Oblique section through crystalliferous idioblast with crystals in regular files. Matrix material stains positively for nucleic acid (RNA) and mucopolysaccharides. Azure-B. X 1,540
Figs. 106-107. Crystals and crystalliferous idioblasts from carpel walls

106. Ultrastructure of edge of crystal fascicle with crystals in regular files. Matrix material is particularly evident around fascicle perimeter. X 7,000

107. Crystal chambers from idioblast with random arrangement of crystals. Matrix material surrounds individual chambers. Arrows denote regions where lamellar nature of sheaths composing individual crystal chambers is evident. Note hexagonal configuration of several chambers. Some distortion may be attributed to heat damage from electron beam. Holes in chambers result from crystals popping out of embedding material during sectioning. X 20,800
Figs. 108-115. Crystalliferous idioblasts from ovary wall. Transsections through idioblast of ovary wall illustrating lamellae which form crystal chambers and of matrix material in which chambers and crystals develop

108. Corner of crystal chamber with lamellae of one facet extending beyond juncture with other facet. Double arrows point out small spherical or tubular structure in matrix material. Other arrows denote row of interlamellar tubules. Tubules at arrow tips appear to have hollow cores, and are of same diameter as tubule in matrix. X 92,400

109. Arrows indicate tubules of matrix in longitudinal and transverse view. X 151,600

110. Small arrows point to matrix tubules in longisectomy; large arrow to intralamellar tubule seen in transsection with electron dense contents. X 222,000

111-112. Arrows indicate matrix tubules in cross- and longitudinal section. Both X 147,200

113-115. Lamellae and matrix material. Note how some lamellae terminate abruptly while others appear to anastamose. Arrow in Fig. 114 denotes matrix tubule in transsection

113. X 224,500

114. X 219,300

115. X 206,800
Figs. 116-125. Crystalliferous idioblasts from ovary wall. Lamellae and surrounding matrix material from chambers in which crystals were removed by catabolic processes. Figs. 116-121 longitudinal, Figs. 122-125 transverse. Note similarities in appearance of longi- and trans-sections, i.e., lamellae terminate and anastamose in both views. Arrows in Figs. 118 and 119 indicate tubules seen in cross-section on crystal (internal) side of sheath.

116. X 87,500
117. X 118,300
118. X 117,000
119. X 117,000
120. X 197,300
121. X 197,300
122. X 282,000
123. X 282,000
124. X 282,000
125. X 282,000
Figs. 126-134. Crystalliferous idioblasts from ovary wall

126-128. Matrix material from idioblast in which crystals have been catabolized. Arrows point to tubules in cross- and longi-section. All X 96,000

129-130. Oblique section of lamellae from two adjacent chambers. Parallel arrangement of tubules is evident. Fig. 130 is enlarged view of sheath from left-hand chamber.

129. X 49,000
130. X 114,000

131-134. Intricacies and complexities of lamellar patterns.

131. X 75,600
132. X 129,400
133. X 47,500
134. X 238,300
Figs. 135-137. Trans-sections of young carpels (early megagametogenesis) demonstrating idioblast differentiation beginning in close association with lateral veins

135. X 105
136. X 252
137. X 252

Figs. 138-139. Acropetal progression of idioblast differentiation. Gynoecium of Fig. 138 with binucleate megagametophytes; of Fig. 139 with nearly mature megagametophytes. Both longitudinal sections X 315
Figs. 140-142. Radial progression of idioblast differentiation. Gynoeicum of Figs. 140 and 142 with binucleate megagametophytes; of Fig. 141 with mature megagametophytes. All trans-sections

140. X 70
141. X 289
142. X 152

Figs. 143-144. Longitudinal sections from gynoeция with mature megagametophytes illustrating idioblast population density and proximity to septal nectary. Both X 473
Figs. 145-147. Early stages of idioblast differentiation

145-146. Proximal sections through young idioblast showing granular nature of cytoplasm at early stage. Note formation of very dense spherical structure. Such structures appear to be centers around which further changes occur.

145. Stained for protein and carbohydrate with napthol black and basic fuchsin. X 1,400

146. Stained for nucleic acids and carbohydrate with methylene-blue-azure II and basic fuchsin. X 1,400

147. Juvenile idioblast illustrating intermediate characters. Evidence of granular cytoplasm yet present, but early signs of adult anatomy visible, i.e., organization into dense mass with arms radiating from it. X 1,400

Figs. 148-150. Illustrations of both types of cytoplasmic patterns: ringed and stellate. Fig. 149 from older gynoecium than Figs. 148 and 150 demonstrating more diffuse nature of cytoplasm in older cells. All sections stained for protein and carbohydrate. Gray material between arms is primarily protein; dense black material primarily carbohydrate.

148. Aniline blue-black and basic fuchsin. X 559

149. Napthol black and basic fuchsin. X 583

150. Aniline blue-black and basic fuchsin. X 559
Figs. 151-154. In ovary wall

151. Scanning electron micrograph of idioblasts. X 293

152. Idioblast with double ring pattern. X 1,400

153. Stellate idioblasts (except ringed idioblast on right) illustrating formation of secondary arms into ring near cell perimeter. Note secondary arms (small arrows) appear to radiate from knobbed terminus of primary arms (large arrows). X 1,800

154. Idioblasts from older carpel demonstrating dissolution of idioblastic substance with age. X 1,700
Figs. 155-166. Cell modifications for material translocation in carpel walls

155-161. Plasmodesmata from walls of cortical cells. Fig. 155 illustrates two that appear to be double. Fig. 161 probably represents a plasmodesma in oblique section. Figure is higher magnification view of structure denoted by small arrow in Fig. 164

155-157. X 99,500
158-160. X 168,000
161. X 170,600

162. Higher magnification view of portion of vesicles in cytoplasm near paramural body; denoted by large arrow in Fig. 164. Note spherical structures in limiting membrane. X 104,000

163. Enlarged view of membranes indicated by arrow in Fig. 166. Circular structures probably represent tubules in cross-section. X 203,000

164. Primary pit field in wall of carpellary cortical cell with an associated paramural body. Note rugose nature of wall. Small arrow points to structure in Fig. 161; large arrow to structure in Fig. 162. X 39,100

165. Wall ingrowth of cortical cell. Note very loose appearance of wall fibrils within ingrowth region. X 33,700

166. Large paramural body containing whorls of membranes with swollen cisternae. X 72,500
Figs. 167-172. Details of nucleus from carpellary cortical cell. Arrow in Fig. 172 points to structure enlarged in Fig. 171

167-170. All X 23,800
171. X 39,000
172. X 17,500

Fig. 173. Nucleus with crystalline inclusion from cortex of carpel. X 10,450
Figs. 174-179. Features of endothelial layer lining locules

174-176. General characters of endothelium (arrows) from gynoecia with mature ovules in Figs. 174 and 175, and nearly mature ovules in Fig. 176

174. X 196
175. X 296
176. X 180

177. Anticlinal division in endothelium from gynoecium with ovules in functional megaspore stage. X 544

Figs. 178-179. Relationship between mature ovules and endothelium in two cases where micropyle opens onto endothelium rather than onto obturator (more common situation)

178. X 489
179. X 1,900
Figs. 180-186. Changes in outer tangential wall of endothelium during carpel dehiscence. Locule oriented toward top of plate in all figures.

180-184. Early stages of dehiscence. Cortical cells and outer epidermis devoid of cytoplasm, cell walls collapsing. Outer wall of endothelium thickening, cytoplasm of endothelium last to degenerate. Cuticular deposits thicken, particularly at cell junctures (Fig. 183, arrow). Endothelium begins to assume undulatory appearance.

180-181. X 139

182. X 327

183. X 638

184. X 310

185. Dehisced carpel. Outer endothelial tangential wall has curled inward into U-shape causing carpels to dehisce along sutures. Note heavy cuticular deposits between cells. X 249

186. Ultrastructure of thick cuticular deposit at outer juncture between two endothelial cells from dehisced carpel. X 23,800
Figs. 187-189. Cytoplasmic remnants of dehisced carpels

187. X 30,000
188. X 28,500
189. X 7,600
Figs. 190-194. Obturator development

190-191. Epidermal cells at base of funiculus divide anticlinally and then elongate forming obturator primordium (arrows). Both X 280

192. SEM illustrating early stage of obturator growth. That portion between ovules is derived from tip of carpel margin only; that adjacent to micropylar side of funiculus from carpel and funiculus. X 260

193-194. Successively older ovules illustrating obturator growth and its relation to micropyle as ovule becomes anatropous. Both X 533
Fig. 195. Median section of fully anatropous ovule with nucellus and megagametophyte removed to show relationship of micropyle to obturator in those ovules where micropyle opens directly onto obturator (usual condition). X 306

Fig. 196. Obturator on ovular side of funiculus. X 319

Fig. 197. Median section through funiculus of two adjacent ovules illustrating obturator on ovular side (upper and lower arrows) and between ovules (center arrow). Portion between ovules can be seen to originate from carpel tips. X 105

Fig. 198. Longi-section of gynoecium showing apical extent of obturator (arrows). X 264

Fig. 199. Section through obturator between ovules. X 300

Fig. 200. Trans-section through upper portion of ovary (at level slightly above arrows in Fig. 198) looking down on uppermost portion of obturator. X 360
Fig. 201. Trans-section of gynoecium with mature ovules awaiting fertilization. Material over top of ovules is silver paint applied to prevent specimen charging while in scanning microscope. X 325
Fig. 202. Top view of obturator between rows of ovules and between adjacent ovules. Point of attachment of two funiculi is evident on left. Secretory product of obturator is evident, particularly in crevices between cells. X 820

Fig. 203. Section through obturator showing columnar shape of individual cells and distal vacuole. Secretory product appears as thin film over top of cells. X 2,940

Figs. 204-205. Pollen tubes growing along obturator surface

204. X 1,470

205. X 2,570
Figs. 206-207. Distal end of obturator cell. Arrow in Fig. 206 indicates secretory product seen at greater magnification in Fig. 207. Note material apparently has two phases: flocculent and dense. Dense phase appears as dark material next to cell wall.

206. X 15,000

207. X 102,000

Fig. 208. Young obturator shortly after initiation of secretory phase. Note warty appearance caused by secretory product occurring as droplets. X 7,200

Figs. 209-210. Pollen tubes growing along surface of obturator. Note change in appearance of secretory product as compared between young stage in Fig. 208 and mature stage in Fig. 210

209. X 942

210. X 5,860
**Fig. 211.** Stylar epidermis and cortex. Numerous lipid droplets in epidermis appear as small dark granules. Note very thick outer epidermal wall. Arrow indicates region where rugose nature of outer wall is evident. X 680

**Figs. 212-217.** Ultrastructural details of outer epidermal wall and cuticle. Note similarities with cuticle from ovarian region. Epicuticular wax does not occur in sheets as in ovary; is uniformly flocculent (Figs. 212, 216, 217). Wall first becomes rugose in outer region of juncture between adjacent cells (Figs. 212, 216)

212. X 75,500
213. X 35,000
214-215. X 60,000
216-217. X 66,200
Fig. 218. Epidermal cells of style. Note rugose nature of outer portion of thick outer wall and small wall invaginations along inner surface of this wall. Cells highly vacuolate with only thin layer of peripheral cytoplasm. X 8,750

Fig. 219. Higher magnification of ramified plasmodesmata in wall of epidermal cells denoted by arrows in Fig. 221. X 30,600

Fig. 220. Higher magnification of cytoplasmic mass in epidermal cell vacuole denoted by large arrow in Fig. 221. X 30,600

Fig. 221. Stylar epidermal cell demonstrating thin arms of cytoplasm which project into and across vacuole. Small transfer-type wall ingrowths occur along one wall and ramified plasmodesmata along another (arrows). X 11,600
Fig. 222. Stylar epidermal cells. Note parallel strands of ER, transfer wall ingrowths, cytoplasmic projections into vacuole which may contain plastids and dictyosomes. Plastids have dense stroma and osmiophilic inclusions. X 30,000
Fig. 223. Trans-section of style demonstrating collateral bundle and part of stylar canal. Note numerous lipid bodies in cortical cells (dark granules). X 357

Figs. 224-225. Cells of stylar cortex illustrating central position of nuclei and secondary vacuole formation by infoldings of tonoplast. Some lipid bodies are evident. Note flocculent contents of vacuoles

224. X 6,000
225. X 7,500
Fig. 226. Cortical cell nucleus. X 13,300

Fig. 227. Cells from cortex of style, upper cell forming a secondary vacuole which contains ER. Flocculent material characteristic of cortical cell vacuoles is apparent in both cells. See also in Fig. 226. X 35,400
Figs. 228-229. Cortical cells from young style tips. Cells in various stages of elongation. Younger, unexpanded cells contain starch and lipid bodies. Flocculent contents of vacuoles are evident in some larger vacuoles. Both X 537

Fig. 230. Cortical cell with numerous spherosomes. Note how those lipid bodies which project into cell vacuole are much less dense in that portion which protrudes into vacuole than in remainder of spherosome. Spherosomes which do not protrude into vacuole are uniformly dense. X 23,300

Fig. 231. Section illustrating dense nature of cortical cytoplasm. X 30,400
Figs. 232-235. Cortical cell nuclei with crystalline inclusions

232. X 14,200
233. X 21,600
234. X 12,800
235. X 11,050
Fig. 236. Cortical cells from old style. Note clusters of many plasmodesmata. X 22,500
Fig. 237. SEM of trans-section through style illustrating secretory material which fills canal. X 260

Fig. 238. Juvenile style prior to secretory activity of cells lining canal. Note empty appearance of these cells relative to same type cells from older styles in Figs. 247-250. X 336

Fig. 239. Pre-secretory stylar canal cells illustrating peripheral nature of cytoplasm and thick cuticle. Note dictyosomes with large vesicles. Two lipid bodies are apparently being catabolized and are extruding into vacuole. Arrows indicate ER strands which project into cytoplasm from lipid bodies. X 24,900
Figs. 240-243. Sloughing of stylar canal cell cuticle antecedent to secretory activity of canal cells. Sloughing progresses centrifugally. Note abundance of lipid droplets in canal cells with cuticle intact and scarcity in cells with cuticle sloughed. Secretory material begins to fill canal as soon as cuticle is sloughed, and stains positively for lipid with osmium. All x 615
Figs. 244-246. Early phases of secretory activity in style

244-245. Positive staining reaction of early secretory product for protein with napthol black. Arrows indicate canal cells that contain lipid bodies, some of which are very large. Such cells are probably just initiating secretory activity. Both X 638

246. Young canal cells at onset of secretory phase. Note ER with highly swollen cisternae. Black globules in secretory product are most likely lipoidal materials. X 12,750
Figs. 247-250. Secretory activity of stylar canal cells. Lipoidal materials accumulate as dense bands in irregular shapes which roughly parallel contours of canal. Proteinaceous materials are mainly confined to center of canal, on inside of lipoidal bands. Carbohydrate substances are apparently not secreted until after other materials have accumulated to some degree. Carbohydrates mainly accumulate around periphery of canal (light gray material outside lipoidal bands) but also occur intermixed with protein moiety. Arrows indicate heavy concentrations of secretory materials along secretory face of canal cells prior to their dispersal in canal proper.

247. X 420
248. X 315
249-250. X 357
Figs. 251-252. Young stylar canal cells at initial phase of secretory activity. Note lipid bodies with halolike appearance implying uniform centripetal catabolism. Numerous lipid globules occur within finely reticulate protein matrix. Large arrows indicate scroll-like polysomes. Small arrows indicate direct ER-plasmalemma connections. Note paramural bodies along secretory face of both cells in Fig. 252.

251. X 23,000
252. X 28,000
Figs. 253-254. ER in young endothelial cells and reticulate protein moiety of secretory material. Some lipoidal material also visible as black globules and as bandlike aggregation.

253. X 34,300

254. X 47,700
Figs. 255-256. Pre-anthesis endothelial cells at intermediate stage of development. RER changed to SER, cells filling with cytoplasm, mitochondria numerous. Proliferation of plastids evident in Fig. 256

255. X 14,200

256. X 22,200
Fig. 257. Three mitochondria from actively secreting canal cell. ER approaches, but does not ensheath them. X 57,900

Fig. 258. Plastid from same cell as mitochondria in Fig. 257. SER ensheaths plastid and appears to touch it (small arrow). Inner plastid membrane invaginates to form large swollen cisternae (large arrows). Plastid also contains starch. X 55,700
Fig. 259. Starch-engorged plastids ensheathed by SER from actively secreting canal cell. Note numerous swollen cisternae and dark osmiophilic inclusions in plastids. X 26,300
Figs. 260-261. Secretorily-active endothelial cells illustrating ensheathment of plastids by SER. Plastids demonstrate invagination of inner membrane to form numerous and often large cisternae. Plastids have abundant lipid inclusions

260. X 28,300

261. X 24,200
Fig. 262. Cell of stylar canal endothelium at peak of synthetic and secretory activity. Note extensive ER, numerous dictyosomes and ribosomes, and cell wall transfer areas. X 10,900

Fig. 263. Enlargement of dictyosome from cell on left in Fig. 62 illustrating their size and numerous vesicles. X 16,700
Figs. 264-266. Endothelial cells

264. Membrane complexes and associated vacuoles possibly derived from organelle degeneration. Enlargement from region indicated by double arrows on Fig. 266. X 12,500

265. Membrane whorls possibly originating from cristae and inner membrane of degenerating mitochondrion. Such configurations are possible precursors to complex depicted in Fig. 264. Single arrow on Fig. 266 indicates position in mother cell. X 18,200

266. Stylar canal cells illustrating lobed nucleus with many nucleoli. Secretory product is evident along secretory face in canal. Note that dense globules are associated with a less dense material. X 8,000
Figs. 267-269. Endothelial cells

267. Enlargement of dictyosome and associated vesicles from Fig. 269. X 90,900

268. High magnification of vesicle being extruded from canal cell into region where cell wall is expanded into transfer zone. X 142,900

269. Stylar canal cells during height of secretory activity. Note large population of ribosomes, extensive ER, plastids with dense stroma and swollen cisternae, and large dictyosomes. Portions of radial wall are expanded to form regions for material transfer. Arrow indicates vesicle being extruded (Fig. 268). Canal oriented toward top of plate. X 35,700
Figs. 270-273. Stylar canal cells

270. Detail of secretory face from region between arrows in Fig. 273. Note vesicles between plasmalemma and wall proper. X 48,800

271. Portion of lobed nucleus and nearby plastids from endothelial cell. X 17,100

272. Large plastid adjacent to region of radial wall expansion. X 15,500

273. Endothelial cell illustrating numerous large dictyosomes with many associated vesicles. Extensive ER, abundant ribosomes, and secretory material along secretory face are evident. Arrows indicate region enlarged in Fig. 270. X 16,700
Fig. 274. Canal cell with expanded region along radial wall. Dense globules of secretory material occur in canal. Note association of blobules with less dense material. X 16,000

Fig. 275. Canal cell with large dictyosomes and numerous vesicles. Mitochondria and ribosomes are numerous also. Some polysomes are scroll-like. Large arrows denote region enlarged in Fig. 276. X 26,250

Fig. 276. Enlargement of region between large arrows in Fig. 275 illustrating abundance of polysomes. Arrow indicates scroll-like configuration and corresponds with small arrow in Fig. 275. X 59,000
Fig. 277. Canal cells with very large wall expansion regions. Arrow indicates polysomes depicted in Fig. 278. X 42,000

Fig. 278. Enlargement of coiled polysomes from Fig. 277. X 168,000
Fig. 279. Stylar canal lined by actively secreting endothelial cells. Note accumulation of lipoidal material into an irregularly shaped band slightly removed from cells. X 6,250

Fig. 280. SEM of secretory product filling canal. Three phases are evident. Carbohydrate moiety is primarily peripheral and appears relatively smooth. Protein moiety appears coarsely granular; occurs primarily in center of canal. Lipid moiety occurs as band between carbohydrate and protein accumulations in some parts of canal. Lipid also occurs as globules as in this micrograph. X 1,365

Fig. 281. Endothelial cell with large accumulation of secretory product in close proximity to secretory face. X 12,100
Figs. 282-286. Development of stigmatic papillae

282. Early stage of papillal differentiation. Epidermal cells over apex of young style expand and elongate. X 460

283. Higher magnification view of young papillae illustrating peripheral nature of cytoplasm and presence of large vacuoles. X 1,438

284. SEM of mature individual papilla showing rugae formed by outer wall. X 2,428

285. Young papillae emerging from stylar apex. X 741

286. Mature stigma seen from below. Elongate form of stylar epidermal cells is evident. Note fuzzy appearance of style due to flocculent nature of epicuticular wax (see Figs. 212 and 217). Papillae near base of stigma are smaller than those on top. X 156
Fig. 287. Stigmal papillae and adjacent cortical cells. Lipid bodies bordering on vacuole appear to become digested on vacuolar side while lipid bodies confined to cytoplasm are homogeneous. Small arrows indicate plasmodesmata in cross-section. Large arrows point to small transfer-type ingrowths. Irregularities in cell wall at right represent early stage of rugae formation. Heavy cuticle surrounds papillae. X 14,600
Fig. 288. Stigmal papilla (left) and epidermal cell (right). Arrows indicate wall transfer regions. Note transfer region in papilla occurs adjacent to region where outer portion of wall has become expanded with fibrils forming a loose reticulum. Compare differences in lipid bodies according to position and parent cell type. X 21,800
Figs. 289-291. Details of papillal cell wall at juncture of papilla and epidermal cell. Wall of papilla consists of two discrete regions. Inner region is more dense while outer region is reticulum of loosely woven fibers. Dark material at left center in Figs. 289 and 290 represents grazing section through cuticle and cell wall of adjacent papilla.

289. X 38,000
290. X 68,000
291. X 98,000
Fig. 292. Basal portion of stigmatic papilla with lobed nucleus and several lipid bodies. X 18,800

Figs. 293-295. Sequence of events following anthesis

293. Arrow indicates just-opened flower. Anthers not yet dehisced; stigma not fully elongated. X 1

294. Flower with anthers opened. X .9

295. Flower receptive for pollination. Small arrows point to nectaries at tepal tips; large arrow to drop of nectar at base of ovary exuded from septal nectary. X 1.1
Fig. 296. Developing fruit with withered style and stigma. X 1.5

Fig. 297. Cell wall and cuticular remnants of outer epidermis from style of dehisced carpel. X 46,200

Fig. 298. Cortex of withered style. Exterior oriented toward top of plate. X 525

Fig. 299. One lobe of withered style and stylar canal. Note cell walls seem to remain more intact at cell junctures around intercellular spaces. Pollen tube remnants persist in canal. X 368

Figs. 300-303. Dehisced carpels. Arrows indicate aborted ovules. All X .9
Figs. 304-309. Integument initiation

304. First indication of integument initiation is swelling in epidermis near base of ovule. Archesporial cell becomes evident at same time. X 1,318

305. Anticlinal divisions in nucellar epidermis produce inner integument primordium. X 626

306-309. Successive anticlinal divisions (Fig. 306, arrow) cause integument to grow around nucellus. Outer integument initiated as inner integument approaches summit of nucellus

306. X 389

307. X 926

308. X 1,146

309. X 850
Figs. 310-314. Integument maturation

310. Outer integument is 3-4 cells high when ovule has turned 90°. X 440

311. Ovules inverted approximately 135°, outer integument has closely approximated extent of inner integument. X 180

312. Fully inverted ovule, both integuments fully extended. X 320

313. Trans-section of ovule. Cells of nucellus proper in center; nucellar epidermis forms single-layered ring of large cells between two small arrows; inner integument comprises next two layers between two large arrows; outer integument consists of outer three cell layers distal to single small arrow. X 334

314. Inner integument is 3-4 cells thick around micropyle. X 500
Fig. 315. Nucleus of integumentary cell. X 7,700

Figs. 316-318. Epicuticular wax deposits between nucellus (right side) and inner integument (left side)

316. Wax first appears as globules. X 2,700

317. Wax later assumes flocculent appearance. X 450

318. Higher magnification view of region denoted by arrow in Fig. 317. X 2,640
Fig. 319. Archesporial cell and parietal cell initial. X 2,288

Fig. 320. Divisions of parietal cell initial result in 6 parietal cells. Mitotic divisions elsewhere in nucellus allow for size increase of nucellus to accommodate expanding MMC. X 649

Fig. 321. Degenerating nucellar cells around periphery of developing megagametophyte allow megagametophyte to expand. Arrow points to cell with intensely staining nucleus which is first stage of cell degeneration. X 578
Fig. 322. Intensely staining nuclei of nucellar cells located between nucellar cap and developing megagametophyte. Such nuclei indicate cell degeneration is imminent. X 1,322

Fig. 323. Trans-section of nucellus between nucellar cap and developing megagametophyte illustrating degenerate nucellar cells. X 1,416

Fig. 324. Trans-section through FM illustrating that degeneration of nucellar cells proceeds centrifugally in all directions. X 1,578

Fig. 325. Degenerate nucellar cells adjacent to micropylar end of 4-nucleate megagametophyte. X 1,647
Figs. 326-329. Megagametophyte expansion and nucellar degeneration

326. Lower ovule illustrates expanding megagametophyte reaching nucellar cap during interphase between first and second mitotic divisions. X 173

327-329. Megagametophytes in late stages of axial extension. Last remnants of degenerating nucellar cells yet remain between megagametophyte and nucellar cap. Note large amyloplasts around nuclei in Fig. 329

327. X 2,615
328. X 1,579
329. X 2,092
Figs. 330-331. Final stage of axial extension of megagametophyte.

330. Dark line between megagametophyte and nucellar cap represents crushed nucellar cells. Note columnar shape and thickened proximal tangential walls of nucellar cap. Thickness of radial walls attenuated distally. X 638

331. All traces of former parietal cells between megagametophyte and nucellar cap are now gone. Thick proximal walls and distal vacuole are characteristic features of nucellar cap. X 1,995

Fig. 332. Meiosis I. Arrows indicate first regions of starch accumulation within ovule. X 340

Fig. 333. Nucellar cap illustrating absence of amyloplasts from nucellar epidermis. Characteristic distal vacuole and thick proximal walls are characteristic. X 1,417

Fig. 334. Cross-section of ovule through proximal end of nucellar cap illustrating thickened radial walls of nucellar cap in this region. X 570

Fig. 335. Longi-section through ovule at FM stage. Micropyle formed only by inner integument with nucellar cap at terminus. Arrows point to material secreted by nucellar cap and inner integument cells. X 413
Fig. 336. Micropyle prior to secretory activity by nucellar cap and inner integument. X 1,273

Figs. 337-339. Secretory activity by nucellar cap

337. Trans-section of ovule through micropyle at a level just distal to nucellar cap. X 762

338. Higher magnification of nucellar cap cells facing micropyle. Cells in early stage of secretory activity indicated by light coating of cells with flocculent material. X 2,500

339. Cells of nucellar cap at stage slightly advanced over those in Fig. 338. X 3,440
Figs. 340-345. Progressive stages of filling of micropyle with secretory material produced by nucellar cap and inner integument where it lines micropyle

340. Initial secretory activity. X 940
341. X 922
342. X 807
343. X 1,017
344. X 1,100
345. Final stage with exostomium covered by thin hymenlike sheet of material. X 940
Figs. 346-349. Post-fertilization micropyle

346. Longisection of micropyle from fertilized ovule. Arrows indicate remains of secretory material adhering to nucellar cap. X 538

347. External view of micropyle from mature seed. Arrow points to funicular scar. X 230

348. Oblique section of mature seed through micropylar area illustrating embryo and plug of flocculent material. X 195

349. Enlargement of flocculent material in micropylar region of mature seed. X 918
Fig. 350. Nucellus near hypostase illustrating high population of amyloplasts in this region of ovule. X 6,600

Figs. 351-353. Pinocytotic vesicles from proximal end of nucellar cap cells

351. X 62,900
352. X 42,400
353. X 43,700
Fig. 354. Nucellar cells chalazad FM, stain slightly more intense than neighboring cells and cell walls begin to thicken denoting onset of hypostase differentiation. X 473

Figs. 355-356. Early basipetal progression of hypostase differentiation from around chalazal end to mid-region of megagametophyte

355. X 1,473
356. X 718

Figs. 357-358. Differentiation of hypostase complete at chalazal end of megagametophyte

357. X 1,558
358. X 126
Fig. 359. Mature megagametophyte illustrating full extent of hypostase (arrows). Note large number of starch grains in nucellus around micropylar end of hypostase. Protoxylem cell of vascular strand is visible in chalaza. X 179

Fig. 360. Mitotic division of chalazal cell (arrow) adjacent to hypostase. X 420

Figs. 361-362. Chalazal cells between hypostase and vascular tissue appear in radial files due to mitotic activity as illustrated in Fig. 360. Both X 390
Figs. 363-364. Examples of ovules with megagametophyte in 4-nucleate (Fig. 363) and 8-nucleate (Fig. 364) stages having cells chalazad hypostase in radial files

363. X 368
364. X 1,569

Figs. 365-366. Degeneration and crushing of nucellar cells at chalazal end of growing binucleate megagametophyte

365. X 380
366. X 1,983
Figs. 367-372. Chalazal end of hypostases. Arrows in Figs. 367-369 and 371 indicate unique cells. Figs. 370 and 372 illustrate hypostases which contain starch but have no unique cells. Hypostases in Figs. 367, 369, and 371 have both starch grains and unique cells. All X 1,658
Fig. 373. Cross-section of ovule through hypostase illustrating two unique cells toward top left and top center. Note very thick walls of hypostase cells. X 5,075
Fig. 374. Hypostase showing presence of only a few starch grains in cell at upper left, but otherwise being generally devoid of contents. Nucellar cells surrounding hypostase (bottom row of cells) contain much starch. X 3,300

Fig. 375. Longisection through chalazal end of ovule showing hypostase, two antipodals, and a polar nucleus. X 1,075

Fig. 376. Cross-section of chalazal end of ovule from flower ready to open. Note density of starch accumulation in nucellar cells around hypostase. X 704
Fig. 377. Early stage of funicular curvature which will eventually result in fully anatropous ovule. Curvature caused by greater number of cell divisions on distal side of funiculus. X 376

Fig. 378. Longi-section of procambial strand in funiculus near chalaza. Arrows indicate cells undergoing paraclinal division. X 595

Fig. 379. Cross-section of funicular procambium, one cell of which is in metaphase of a paraclinal division. X 1,537

Fig. 380. Paraclinal division of procambial cell in chalaza of ovule with binucleate megagametophyte. X 413
Fig. 381. Median longitudinal section of ovule illustrating course of vascular tissue from placenta to chalaza. Arrow indicates dividing cell illustrated in Fig. 380. X 168

Fig. 382. Differentiating protoxylem cell in chalaza. X 586

Fig. 383. SEM of terminal protoxylem cell in chalaza (arrow). X 480

Fig. 384. Differentiating protoxylem cell at bend of funiculus. Note highly elongate nucleus with many nucleoli. X 1,488

Fig. 385. SEM trans-section of funicular vasculature. Arrow indicates protoxylem cell with annular thickenings. X 1,000

Fig. 386. Protoxylem cells in chalaza (arrows). Vertical arrow denotes terminus of vascular strand. Terminal cell is commonly short (see also Fig. 383). X 403
Figs. 387-391. Archespore differentiation

387. Nucellus prior to archespore differentiation. X 1,417

388. Longitudinal section through ovary with ovules at archesporial cell stage. Style oriented to right. X 140

389-391. Examples of archesporial cells stained for protein and carbohydrate with aniline blue black and basic fuchsin

389. X 1,527

390. X 1,870

391. X 2,125
Figs. 392-393. Archesporial cells stained for nucleic acids and carbohydrate with methylene blue-azure II and basic fuchsin

392. X 1,493
393. X 1,875

Fig. 394. Cross-section of MMC just prior to meiosis. X 1,955

Fig. 395. Cross-section of MMC during meiotic prophase I. X 1,955
Fig. 396. Longitudinal section of ovule during megaspore mother cell stage. Nucellar epidermis clearly differentiated into juvenile nucellar cap. Note already-thickened tangential wall between parietal cells and nucellar cap, and proximally thickened radial walls of nucellar cap. Cells around chalazal end of MMC in early stage of degeneration. X 1,252
Fig. 397. Megaspore mother cell. X 2,800

Fig. 398. Ovule at MMC stage. Nucellus becoming more vacuolate relative to younger stages. Cells at apex of nucellar epidermis elongating to form nucellar cap. Wall between nucellar cap and nucellus proper thicker than other walls. Ovule has four parietal cells. X 420

Fig. 399. Ovules at MMC stage turned 90°. Upper ovule has six parietal cells; lower has four. Nucellar cap and its cell walls are distinct in both. X 210
Figs. 400-401. Linear tetrad of haploid megaspores resulting from meiosis. Chalazal pair is larger than micropylar pair; and of chalazal pair, chalazalmost is largest. Most instances this one becomes functional megaspore. Note staining differences between functional megaspore nucleus and nucleus of third megaspore. Adjacent sections

400. Aniline blue-black and basic fuchsin. X 1,208

401. Methylene blue-azure II and basic fuchsin. X 1,208
Figs. 402-403. Growth of functional megaspore and degeneration of non-functional megaspores. Functional megaspore generally produces two large polar vacuoles with nucleus centrally positioned. Vacuoles of nonfunctional megaspores usually coalesce into one large vacuole as in megaspore below FM in Fig. 402. Megaspores in advanced stages of degeneration appear as dark masses. Both X 670

Fig. 404. Functional megaspore with two polar vacuoles. Staining for protein reveals numerous spherical and rod-shaped cytoplasmic contents. Aniline blue-black and basic fuchsin. X 1,470

Fig. 405. Functional megaspore with only one polar (micropylar) vacuole. X 1,470

Fig. 406. Metaphase of first mitotic division initiating megagametogenesis. Note perinuclear organization and equal distribution of amyloplasts and other organelles. X 1,437
Figs. 407-411. Bimucleate megagametophytes. Following first mitotic division, nuclei migrate to opposite poles, cytoplasm thickens and vacuole forms in center of megagametophyte between nuclei. Organelles reassume perinuclear orientation. Some large amyloplasts may contain several starch grains (Fig. 411)

407. X 551
408. X 417
409. X 1,569
410. X 1,542
411. X 1,653

Figs. 412-413. Tetranucleate megagametophytes.

412. Nuclei in linear array which is most common condition. Note perinuclear distribution of organelles. X 284

413. Variation in nuclear distribution pattern from usual linear array. Numerous starch grains are present in micropylar end of nucellus. X 284
Fig. 414. Cross-section through tetranucleate ovule in which both pairs of nuclei are located side by side, vice linearly, at their respective poles. Pair illustrated are from micropylar pole. Note perinuclear distribution of organelles. X 1,593

Fig. 415. Tetranucleate megagametophyte illustrating perinuclear organelle distribution. Note greater number of organelles around micropylarmost nucleus, particularly on its micropylar side. X 400

Fig. 416. Apportionment of organelles between pair of recently-formed chalazal nuclei in tetranucleate megagametophyte. Organelles move with separating nuclei. X 1,625

Fig. 417. Separation of micropylar nuclei. Organelle distribution is uneven with micropylarmost nucleus retaining more than its sister nucleus. X 1,625
Fig. 418. Egg apparatus consisting of egg and two synergids with prominent filiform apparatus. Portion of cytoplasmic strand which traverses central cell between egg apparatus and antipodals is visible. X 880

Fig. 419. Top view of egg apparatus from level of antipodals. Close inspection will reveal lobe formed by egg at lower right, and two larger lobes formed by synergids. Cytoplasmic strand is also present. X 1,183

Fig. 420. Antipodals and chalazal polar nucleus. Note abundance of starch in nucellus around hypostase. X 874

Fig. 421. One synergid and cytoplasmic strand. X 2,933
Figs. 422-425. Polar nuclei and cytoplasmic strand

422. Narrow cytoplasmic strand (arrow) and one freeze-fractured polar nucleus. X 1,590

423. Massive cytoplasmic strand. X 2,470

424. Both polar nuclei and cytoplasmic strand. Staining for protein reveals numerous spherical, lenticular, and elongate structures. Strand appears to be an aggregation of several smaller strands. X 1,842

425. Juxtaposition of polar nuclei at chalazal end of central cell adjacent to antipodals. X 765
Fig. 426. Longi-section through three ovules. Large cavity in ovule on right is vacuole of central cell which occupies most of that cell's volume. Antipodals are visible just above central cell. X 400

Figs. 427-428. Cytoplasm of central cell revealing presence of many amyloplasts

427. X 5,140

428. X 1,800
Figs. 429-433. Antipodals from 8-nucleate megagametophytes

429. Transverse fracture of ovule showing hypostase and chalazalmost antipodal at vacuolate stage. X 1,050

430. Transverse fracture of ovule at same level as ovule in Fig. 429 but at later developmental stage. Note antipodal is now filled with cytoplasm. X 860

431. Chalazal antipodal early in transition from being vacuolate to stage in which it is filled with cytoplasm and starch grains. X 2,125

432. Higher magnification view looking down into vacuolate antipodal. X 2,000

433. Longi-section of chalazal antipodal after filling with cytoplasm. Numerous starch grains are discernible. X 780
Fig. 434. Median longitudinal section of mature egg apparatus stained for protein and carbohydrate. Cell wall appears to be absent over chalazal side of egg and very lightly staining between egg and synergid. Wall around synergid stains intensely with basic fuchsia except where common with egg. Note difference in staining properties of synergid wall and nucellus cap wall where they are common along base of synergid. Micropylar synergid wall has proliferated to form prominent filiform apparatus which stains predominantly for carbohydrate. Black or very dark projections of FA stained positively for protein. Reaction of protein staining also diffuse throughout FA. Wall in hook region forms thickened structure with knobbed terminus. This structure appears at base of outer wall of each synergid. Synergid cytoplasm has very coarsely granular texture as does that of egg. Note difference in morphology and staining reactions of egg and synergid nuclei. Synergid nucleus very large with nucleolus almost same size as entire egg nucleus. Also note very large pars morpha phase of nucleolus. Characters of synergid nucleus indicative of secretory active cell. Small segment of cytoplasmic strand projects upward from egg. Aniline blue black and basic fuchsia. X 2,390
Fig. 435. Oblique fracture through micropylar end of ovule illustrating position of egg apparatus relative to ovule. X 400

Fig. 436. One synergid and egg. Basal vacuole and apical position of nucleus in egg are evident. Note that egg is in contact with synergid laterally and only apical portion of egg projects above synergid. X 1,680

Fig. 437. Higher magnification view of egg apparatus in Fig. 435 illustrating lateral extent of filiform apparatus. Pollen tubes enter synergids from this direction. X 2,000
Fig. 438. Longisection of ovule with immature egg apparatus; only one synergid visible. Characteristic position of vacuoles well-illustrated; chalazal in synergid (cell on left), micropylar in egg (cell with nucleus on right). Contact between egg and nucellar cap clearly evident. X 240

Fig. 439. Longisection of ovule illustrating both synergids, chalazal polar nucleus, and two antipodals. Note chalazal position of vacuoles in synergids and obvious lack of filiform apparatus even at such an advanced stage of megagametophyte maturity. X 315

Fig. 440. Egg apparatus with most of egg removed by fracture. Cytoplasm under label (E) is most likely from region very close to egg nucleus. Basal vacuole of egg appears as cavity under this cytoplasm. Large lobes represent synergids, each of which has a cytoplasmic strand connected to it. X 2,482
Fig. 441. Median longitudinal section of synergids prior to filiform apparatus initiation. Differences in wall characters between gametophyte and sporophyte are evident. Note size of synergid nucleus and of pars morpha phase of nucleolus. Section stained for nucleic acids and carbohydrate with methylene blue-azure II and basic fuchsin. X 2,200

Fig. 442. Cross-section of egg apparatus with developing filiform apparatus. Note occurrence of several vacuoles around periphery of FA. Also note nuclear size and characters. Perimeter of large apical vacuole appears along upper margin. Methylene blue-azure II and basic fuchsin. X 2,460

Fig. 443. Median longitudinal section of filiform apparatus stained for nucleic acids and carbohydrate. Black granules in FA are intensely staining carbohydrate entities while dark staining region along FA perimeter reacts strongly with both carbohydrate and nucleic acid dyes. Note conspicuous intercellular space at juncture of basal ends of FA and nucellar cap. Slight gradient in density of cytoplasm evident from apical to basal ends of synergid. Methylene blue-azure II and basic fuchsin. X 3,140
Fig. 444. Clusters of plastids from chalazal end of synergid. Note lack of starch. X 14,000

Figs. 445-446. Mitochondria with large swollen cristae are abundant throughout synergid cytoplasm. Portion of filiform apparatus is visible. Note connection of ER with plasmalemma along FA (arrow). Dictyosomes and microbodies are also present

445. X 33,600

446. X 28,000
Figs. 447-448. Synergid cytoplasm illustrating numerous dictyosomes and much ER. Note periodic expansions along synergid-central cell cell wall in Fig. 447

447. X 41,200

448. X 31,500
Figs. 449-451. Synergid cytoplasm in relation to filiform apparatus. Dictyosomes with numerous vesicles are commonly found in pockets and channels of cytoplasm formed by labyrinthine nature of FA. Several vesicles with some sort of fibrillar or flocculent contents appear to be included within FA wall material.

449. X 45,750
450. X 50,000
451. X 36,000
Fig. 452. Chalazal portion of synergid illustrating large amount of ER present in this region of cell. Central cell-synergid wall is visible near top of micrograph. X 74,400

Fig. 453. Filiform apparatus demonstrating complex nature of cell wall. Note several different phases of wall and presence of vesicles within wall. X 54,000
Fig. 454. Initial stage of filiform apparatus formation. X 2,040

Fig. 455. High magnification view of knobbed wall structure from base of peripheral synergid wall. X 2,975

Fig. 456. SEM of filiform apparatus. Note coarsely granular texture. X 1,964

Fig. 457. Synergids and filiform apparatus. Vacuoles around FA and density of synergid cytoplasm are apparent. X 2,700
Figs. 458-463. Binucellate ovules, both with MMC at meiotic dyad stage

458-459. Normal ovule on left, binucellate ovule on right. Both X 168

460-461. Dyad of binucellate ovule stage. Both X 315

462. Functional megaspore from normal (left) ovule in Fig. 458. X 1,260

463. Functional megaspore from normal (left) ovule in Fig. 459. X 1,260
Figs. 464-469. Serial sections through normal (left) and binucellate ovules

464. Normal (left) and binucellate (right) ovules. X 202

465. Megaspore dyad from right-hand nucellus of binucellate ovule. X 1,346

466. Normal (left) and binucellate (right) ovules. X 202

467. Chalazal megaspore of meiotic dyad from left-hand nucellus of binucellate ovule. X 1,346

468. Normal (left) and binucellate (right) ovules. X 202

469. Micropylar megaspore of meiotic dyad from right-hand nucellus of binucellate ovule. X 1,346
APPENDIX C. FORMULAE AND SCHEDULES

I. Fixation, dehydration, and embedding schedules—LM and TEM

A. Paraffin processing (Sass, 1958)

1. Dissection—Whole buds and whole flowers were cut off the parent plant at the pedicular node with a razor blade. Smaller buds were fixed whole, while larger buds and flowers were trimmed down to the gynoecium and the entire gynoecium was fixed. Specimens were put into 10 dram vials for processing.

2. Fixation—Whole buds or entire gynoecia were fixed overnight in FAA.

3. Dehydration
   a. 50% ETOH--15 min
   b. 75% ETOH--15 min
   c. 100% ETOH--2x; 15 min
   d. 1:1 ETOH/xylene--15 min
   e. xylene--2x; 15 min

4. Infiltration and embedding
   a. chill xylene in refrigerator
   b. add melted 61 C wax slowly so that it flows across cold xylene and solidifies.
   c. place in 45 C oven for 3-4 d--swirl vial gently on occasion--xylene is saturated when schlieren lines are no longer produced by swirling.
   d. place in 65 C oven until all wax is melted
   e. pour off ½ of melted wax and refill with fresh wax
   f. repeat #e 3-4 times over 24 h
   g. Pour off all wax and replace with fresh wax--24 h
   h. pour wax and pieces of tissue into mold while applying heat with hot plate or other source
   i. arrange pieces as desired
   j. solidify wax in ice water

5. Sectioning and mounting sections
   a. cut sections 8 μm in thickness with rotary microtome
   b. expand sections by floating on hot water bath (45-50 C)
   c. mount sections on glass slides with Haupt's adhesive
   d. dry slides on warming tray (45-50 C)--0.5-1 h
6. Staining and coverslipping

a. xylene--3x; 10 min each
b. 1:1 xylene/ETOH--10 min
c. 100% ETOH--10 min
d. 75% ETOH--10 min
e. 50% ETOH--10 min
f. 25% ETOH--10 min
g. tap water--10 min
h. 1% safranin 0--overnight
i. tap water--rinse
j. 25% ETOH--10 min
k. 50% ETOH--10 min
l. 75% ETOH--10 min
m. 100% ETOH--10 min
n. 1% fast green in 100% ETOH--10-15 sec
o. 100% ETOH--rinse
p. 1:1 ETOH/xylene--10 min
q. xylene 3x; 10 min each
r. Piccolyte and coverslip
s. place on warming tray (45-50 C) overnight with lead weight on coverslip

B. Resin processing--LM

1. Dissection—Whole buds (for younger stages) and whole flowers (for older stages) were cut off the parent plant at the pedicular node with a razor blade. The buds and flowers were then further dissected on discs of blotter paper saturated with unbuffered 1% glutaraldehyde. Specimens were put into 10 dram vials for processing

2. Fixation and dehydration

a. 3% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.2) at 4 C overnight
b. buffer rinse--4x; 15 min each
c. 25% ETOH--15 min
d. 50% ETOH--15 min
e. 75% ETOH--15 min
f. 95% ETOH--15 min
g. 100% ETOH--3x; 20 min each

3. Infiltration and embedding (Spurr, 1969)

a. 100% acetone--3x; 20 min each
b. 2 acetone:1 resin--15 min
c. 1 acetone:1 resin--30 min
d. 1 acetone:2 resin--1 h
e. resin--overnight on rotator
f. drain pieces on paper towel
g. put pieces into fresh resin in molds
h. cure 24 h at 60 C

C. Resin processing—TEM

1. Dissection—Whole buds (for younger stages) and whole flowers (for older stages) were cut off the parent plant at the pedicullar node with a razor blade. The buds and flowers were then further dissected on discs of blotter paper saturated with unbuffered 1% glutaraldehyde. Specimens were put into 10 dram vials for processing

2. Fixation and dehydration (Horner, 1976)
   a. 2% glutaraldehyde in 0.5 M sodium cacodylate buffer (pH 8.0) at room temperature--1 h
   b. fresh 2% glutaraldehyde in 0.5 M sodium cacodylate buffer (pH 8.0) at 4 C overnight
   c. buffer rinse--4x; 15 min each (room temperature)
   d. 1% OsO₄ in 0.5 M sodium cacodylate (pH 8.0)--2 h
   e. buffer rinse--4x; 15 min each
   f. 1% unbuffered uranyl acetate--2 h
   g. 12.5% ETOH--15 min
   h. 25% ETOH--15 min
   i. 50% ETOH--15 min
   j. 75% ETOH--15 min
   k. 90% ETOH--15 min
   l. 100% ETOH--3x; 20 min each

3. Infiltration and embedding; Epon 812 Resin Mixture (Luft, 1961)
   a. propylene oxide--3x; 20 min each
   b. 1 propylene oxide:1 resin--3 h
   c. pure resin--overnight
   d. drain on paper towel
   e. put into fresh resin in molds
   f. cure 48 h at 60 C

II. Staining schedules for resin embedded sections

A. Light microscopy

1. acid fuchsin (Feder and O'Brien, 1968)
   a. 1% aqueous acid fuchsin--2 h at 60 C
   b. rinse slide with stream of D. H₂O
   c. air dry
2. aniline blue (Currier, 1957)
   a. 0.005% aniline blue in 0.15 M phosphate buffer (pH 8.2)
      --15 min
   b. coverslip as wet mount
   c. use barrier filter 430 nm, and lamphousing filters
      BG 38 and UC 1

3. aniline blue black (Fisher, 1968)
   a. 1% aniline blue black in 7% acetic acid--0.5-1 h at
      60 C
   b. rinse with stream of D. H2O
   c. dry on warming tray

4. azure B (Flax and Himes, 1952)
   a. 1% azure B in citrate buffer (pH 4.0)--4 h at 60 C
   b. rinse with stream of D. H2O
   c. dry on warming tray

5. basic fuchsin (Humphrey and Pittman, 1974)
   a. 0.05% basic fuchsin in 2.5% ETOH--0.5-1 h
   b. rinse with stream D. H2O
   c. air dry

6. methylene blue-azure II (Humphrey and Pittman, 1974)
   a. 0.013% methylene blue-0.002% azure II in phosphate
      buffer (pH 6.9)--1 h at 60 C
   b. rinse with stream of D. H2O
   c. dry on warming tray

7. naphthol black
   a. 1% naphthol black in 7% acetic acid--0.5 h at 60 C
   b. rinse in stream of D. H2O
   c. dry on warming tray

8. Paragon (Spurlock et al., 1966)
   a. form puddle on slide on warming tray--50-55 C until
      edge of puddle begins to dry
   b. rinse with stream of D. H2O
   c. dry on warming tray
9. toolidine blue O (Trump et al., 1961)
   a. 1% toolidine blue O in acetate buffer (pH 4.4)—4 h at 60 C
   b. rinse with stream of D. H₂O
   c. dry on warming tray

B. Transmission electron microscopy

1. uranyl acetate (Watson, 1958)
   a. 30% uranyl acetate in 100% methanol—30 min
   b. 100% methanol—50 dips
   c. 100% ETCH #1—50 dips
   d. 100% ETCH #2—50 dips
   e. 75% ETOH—50 dips
   f. 50% ETOH—50 dips
   g. DD. H₂O #1—50 dips
   h. DD. H₂O #2—50 dips
   i. air dry

2. lead citrate (Reynolds, 1963)
   a. lead citrate—1 h
   b. DD. H₂O #1—50 dips
   c. DD. H₂O #2—50 dips
   d. DD. H₂O #3—50 dips
   e. DD. H₂O #4—50 dips
   f. DD. H₂O #5—50 dips
   g. air dry

III. Scanning electron microscopy processing schedules

A. Dissection—Samples for SEM were cut off the parent plant and dissected under conditions similar to those for LM. For SEM, however, dissection was limited to removing the gynoecium from the floral bud or flower and then cutting off the style at its base. Styles and ovaries were processed separately in 10 dram vials

B. Fixation and dehydration (Horner, 1976)

1. 3% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.2)
2. at 4 C overnight
3. buffer rinse—4x; 15 min each
4. 1% OsO₄ in 0.5 M sodium cacodylate buffer (pH 7.2)—2 h
5. buffer rinse—4x; 15 min each
6. 25% ETOH—15 min
7. 50% ETOH—15 min
8. 75% ETOH--15 min
9. 95% ETOH--15 min
10. 100% ETOH--3x; 20 min each
11. cryofracture or critical point dry

C. Cryofracture

1. put dehydrated pieces into plastic sleeve filled with 100% ETOH
2. drop sleeve into liquid nitrogen (LN) for 2-3 min
3. put frozen sleeve on top of a precooled metal block in LN, so that LN just covers block
4. fracture sample with a sharp blow with a razor blade
5. put fractured pieces back into 100% ETOH to thaw

D. Critical point drying (Horner, 1976)

1. 2 ETOH:1 Freon 113--5 min
2. 1 ETOH:1 Freon 113--5 min
3. 1 ETOH:2 Freon 113--5 min
4. Freon 113--3x; 15 min each
5. put into critical point apparatus
6. 5-6 flushes of system with CO2
7. take through critical point of CO2 (31 C, 1072 psi)
8. mount or store in dessicator

E. Mounting--Specimens were mounted on brass discs with metal tape and conducting paint (Ladd Research Industries)

F. Coating (Horner, 1976)

1. put mounted specimens in vacuum evaporator approximately 18 cm from electrodes
2. evaporate 1 carbon rod
3. evaporate 25.6 cm of 8 mil gold wire
4. remove from evaporator and store in dessicator

IV. Formulae for buffers, fixatives, and stains

A. Buffers

1. Acetate buffer; pH 4.4 (for toluidine blue 0)
   a. 1.35 g sodium acetate/100 ml H2O
   b. 0.6 ml glacial acetic acid/100 ml H2O
   c. 62 ml (a) + 38 ml (b)
2. Citrate buffer; pH 4.0 (for azure B)
   a. 4.2 g citric acid/100 ml H₂O
   b. 5.9 g sodium citrate/100 H₂O
   c. 65 ml (a) + 35 ml (b)

3. Phosphate buffers
   a. 0.06 M; pH 6.9 (for methylene blue-azurell)
      1). 0.907 g monobasic potassium phosphate/100 ml H₂O
      2). 1.188 g dibasic sodium phosphate/100 ml H₂O
      3). 50 ml (1) + 50 ml (2)
   b. 0.1 M; pH 7.2 (for fixation)
      1). 13.609 g monobasic potassium phosphate/liter H₂O
      2). 14.190 g dibasic sodium phosphate/liter H₂O
      3). 110 ml (1) + 390 ml (2)
   c. 0.15 M; pH 8.2 (for aniline blue)
      1). 0.946 g dibasic potassium phosphate/100 ml H₂O

4. Sodium cacodylate buffer; 0.1 M; pH 7.2 (for fixation)
   a. 15.998 g anhydrous sodium cacodylate/100 ml H₂O
   b. 0.83 ml concentrated HCl/100 ml H₂O
   c. 100 ml (a) + 8.3 ml (b) + 91.7 ml H₂O

B. Fixatives

1. FAA (Formaldehyde-Alcohol-Acetic acid)
   a. 50% ETOH--900 ml
   b. glacial acetic acid--50 ml
   c. 40% formaldehyde--50 ml

2. Glutaraldehyde
   a. 2% in 0.05 M buffer
      1). 24 ml stock 0.1 M buffer
      2). 24 ml D. H₂O
      3). 2 ml 50% glutaraldehyde
   b. 3% in 0.05 M buffer
      1). 23.5 ml stock 0.1 M buffer
      2). 23.5 ml D. H₂O
      3). 3 ml 50% glutaraldehyde
3. Osmium tetroxide
   a. 2% stock solution
      1). 0.5 g OsO$_4$
      2). 25 ml H$_2$O
   b. 1% working solution in 0.05 M buffer
      1). 5 ml stock 0.1 M buffer
      2). 5 ml 2% stock OsO$_4$

4. Uranyl acetate
   a. 1 g uranyl acetate
   b. 100 ml H$_2$O

C. Stains
1. acid fuchsin
   a. 1 g acid fuchsin
   b. 100 ml H$_2$O

2. aniline blue
   a. 5 mg aniline blue
   b. 100 ml 0.15 M phosphate buffer, pH 8.2

3. aniline blue black
   a. 1 g aniline blue black
   b. 100 ml 7% acetic acid

4. azure B
   a. 1 g azure B
   b. 100 ml citrate buffer, pH 4.0

5. basic fuchsin
   a. 0.05 g basic fuchsin
   b. 100 ml 2.5% ETOH

6. methylene blue-azure II
   a. 0.13 g methylene blue
   b. 0.02 g azure II or azure A
   c. 10 ml glycerol
d. 10 ml methanol  
e. 30 ml 0.066 M phosphate buffer, pH 6.9  
f. 50 ml H₂O  

7. Naphthol black  
   a. 1 g naphthol black  
   b. 100 ml 7% acetic acid  

   a. 0.73 g toluidine blue 0  
   b. 0.27 g basic fuchsin  
   c. 100 ml 30% ETOH  

9. Toluidine blue 0  
   a. 1 g toluidine blue 0  
   b. 100 ml acetate buffer, pH 4.4  

10. Uranyl acetate  
   a. 15 g uranyl acetate  
   b. 50 ml methanol  

11. Lead citrate  
   a. 1.33 g lead nitrate  
   b. 1.76 g sodium citrate  
   c. 30 ml CO₂-free H₂O  
   d. stir and let stand 30 min with intermittent mixing  
   e. 8 ml 1.0 N NaOH added slowly  
   f. 12 ml CO₂-free H₂O
APPENDIX D. BINUCELLATE OVULE

In two separate ovaries, an anomalous condition was noted in which two nucelli were enclosed within one pair of integuments (Fig. 458). Each nucellus contained a developing megagametophyte with both at the same stage of development (Figs. 458, 459, 464, 466, 468). At the time of fixation, they were in the dyad stage between meiosis I and II.

Measured across the chalaza, the binucellate ovule is approximately one-third again as wide as normal ovules, but there is no difference in length (Fig. 464). Each of the twin nucelli is just slightly narrower and shorter than normal, but the common chalaza is more massive (Fig. 464). In all other aspects, the binucellate ovule appears normal, including general shape and appearance, starch content, and degeneration of nucellar cells around the developing gametophyte (Figs. 458-469).