THE ANATOMY OF ARACHIS HYPOGAEA L.

by

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I

INTRODUCTION

The plant with which this thesis is concerned is Arachis hypogaea L. Hermann (1954) listed nine species of Arachis. Eight of these species are wild and are found only in South America. Arachis hypogaea is known only in cultivation and as an occasional escapee. Hermann (1954) described four forms of Arachis hypogaea. The Jumbo, the Virginia Runner, and the Virginia Bunch are variants of the form macrocarpa and constitute the bulk of peanuts grown in Virginia. For this reason all experimental work was performed with material from plants of Arachis hypogaea f. macrocarpa (A. Chev.) Hoehne.

Interest in the anatomy of the fruit of Arachis hypogaea originally developed at V.P.I. in 1951. At this time an experimental drier was undergoing tests at the Tidewater Field Station of the Virginia Agricultural Experiment Station, Holland, Virginia. In the harvesting of peanuts it is necessary to stack the peanuts and peanut-vine hay in the field for a period of 4 to 8 weeks. This is done in order that the fruits may properly "cure" or dry and subsequently be ready for market or storage (Baker, et al 1952). Naturally there is some loss of fruits to small animals, birds, fungi, adverse weather conditions, etc. during this time. Obviously it would be expedient to the peanut farmer to entirely eliminate this period of exposure. With this in mind, an artificial drier was designed and constructed. Cannon (1951) reported on the design, construction, and functioning of this drier.

Experimentation with the drier in 1951 indicated that a higher percentage of seeds with damaged seed coats was found in the artificially dried fruits than in field dried fruits (Cannon 1951). The damage was described as a breaking or splitting of the seed coat and consequent slippage on the cotyledons. This damage is considered a serious matter, since the value of the peanuts on the market is considerably lowered. A group of peanuts with a high percentage of slippage cannot be sold for use as roasted or salted peanuts, but must be sold for use in oil manufacture. It has been shown (Cannon 1951) that a peanut whose protective seed coat has been split may more readily acquire undesirable off-flavors from the surrounding environment. The quality of the peanut is further lowered by the fact that without the seed coat the cotyledons may more readily separate and break apart, making it undesirable for some purposes.

Members of the staff of the Tidewater Field Station, in an attempt to determine the cause of and possible remedy for the damaging effects of the drier's action, consulted with members of the V.P.I. Biology Department. Since the literature concerning the anatomy of the mature peanut fruit is limited, it was impossible to undertake studies concerning the damage to the seed coat without a more thorough investigation of the anatomy of the structures involved. The objective of this thesis is to provide a basic study of the anatomy of the mature fruit of Arachis hypogaea and to give an outline of the methods used in the study. Coupled with a thorough survey of the literature, this anatomical study and outline of methods should prove useful in future studies

related to an explanation of seed coat damage caused by the artificial drier. It is hoped that the work may also be of value in pathological and parasitological work with the peanut fruit. In addition to a description of the methods used in the study of the various structures and a description of the anatomy of the mature fruit, the anatomy of the mature stem, root, and leaf of the plant is described, as are the anatomical features of the flower and immature fruit. Observations from studies of the germinating seeds are also recorded.

II

REVIEW OF LITERATURE

The literature concerning Arachis hypogaea is rather voluminous when one considers all of the works that have been published dealing with its cultivation, physiology, and economic uses. However, such a copious supply of useful material cannot be found if one is interested in the anatomy of the plant. Much of the work that has been done with Arachis along anatomical lines is quite old and consequently lacking in modern anatomical approach as regards technique, concepts, and detailed examination of various structures. Since the literature does contain so much work concerning non-anatomical studies, it has been necessary to limit the survey of literature in this thesis to papers dealing with the anatomy of the plant or to papers providing pertinent information about anatomical or morphological aspects of various structures.

Pettit (1895) provided the first useful anatomical approach to a study of Arachis hypogaea, although there were several works concerning

the taxonomy, morphology, and growth habits of the plant published prior to her work. The study was undertaken for the purpose of discovering some additional facts concerning the hypogaean development of the fruit. Strongest emphasis is therefore placed on the study of the anatomy and development of the gynophore, although a history of the earlier work is presented, along with a general description of the plant. The descriptions provided are essentially accurate, except for an error in the description of the nyctotrophic movements of the leaves. Pettit states that "The leaflets twist downward and backward so that the lower surfaces of each pair are applied to each other". This erroneous observation is reported correctly in a later paper by Reed (1924) in which he states that the leaflets move upward so that the upper surfaces of each pair are applied to each other. The written description of the anatomy of the developing and mature gynophore is also essentially accurate, but because the work is quite old, the terms employed are rather outdated, as are the concepts concerning several involved structures. The diagrams are accurate, but they are limited in detail and material covered.

A paper by Richter (1899) is probably a reference of value in any study of Arachis hypogaea. Unfortunately this paper has not been available, hence knowledge of it is limited to the work of other authors (Waldron 1918, Smith 1950, Jacobs 1947, Yarbrough 1949). The paper is apparently in a publication that is not readily available in most libraries and no abstracts of the work have been located. Evidently the work covers a number of aspects of a study of Arachis hypogaea plants

grown at Breslau. Due to the fact that the environmental conditions in that region are not ideal for the normal growth of the plant, Smith (1950) points out that some of the data are unreliable, especially that concerning the fertility of the flowers.

Winton (1904) published what appears to be the first useful anatomical research data concerning the mature peanut fruit. Essentially the paper reports on the results of a histological study of the mature fruit, undertaken especially to secure data for use in the microscopical examinations of peanut products. The paper presents an interesting anatomical study of the mature fruit and contains some accurate diagrams of structures and cell types. The descriptions are essentially accurate and fairly complete, considering the concise nature of the report. Because the work was done with the identification of cell types in peanut products as its goal however, a more modern and complete approach is required to add necessary information to that provided. In his work, Winton cites six references dealing with the anatomy of the seed and fruit of Arachis hypogaea that have not been examined by this author, because they were not available in the V.P.I. Library. These papers were the work of Moeller (1886), Vogl (1899), Hanausek (1901 and 1903), Kobus (1884), Uhlitzsch (1892), and Bohmer (1893).

Waldron (1919) published the results of a study that covers the anatomy of Arachis hypogaea more completely than any previous work. His study included the history, general morphology, histology, physiology, and uses of the plant. The section entitled "Histology" includes written

anatomical descriptions of the root, stem, leaf, and fruit of the plant. The descriptions given of the root, stem, and leaf are quite brief and the diagrams are lacking in detail. The anatomy of the fruit is studied in five phases; the anatomy of the young gynophore, the anatomy of the mature gynophore, the anatomy of the young fruit, the anatomy of the developing fruit, and the anatomy of the mature fruit. More modern anatomical terminology and concepts are found in this paper than has heretofore been observed and the descriptions are generally accurate. It is necessary to note however, that Waldron states that "The peduncle, after flowering, lengthens and sinks into the soil carrying the head with it". Actually, as Smith (1950) has pointed out, it is the ovary that elongates and the flowers are sessile, having no peduncles. Waldron demonstrated that root hairs were present on the plant in rosettes at and on the base of side roots and rarely in the region of lateral root tips, although Pettit (1895) and Richter (1899) failed to find any (possibly due to a difference in growth conditions). Waldron stated that their growth is stimulated by a high temperature and humidity. According to Yarbrough (1949), Badami (1935) also noted that root hairs were developed to a limited extent under these special conditions. Waldron further stated that saturated and heavy soil conditions retarded the growth of the rosette type and inhibited the appearance of the tip hairs. Concerning root hairs, Reed (1924) stated "I have been unable to find hairs on the root tips, and but few rosettes on field grown plants, and, since the conditions under which Waldron was able to produce them are

seldom met with in the field, it is doubtful whether they are normally produced in crop cultivation ...". Yarbrough (1949) confirmed the results of these workers with material germinated in a moist chamber and even observed their formation very infrequently on the primary root. He stated that all hairs were very short (1-2 mm. long) and sometimes observed them to be branched, forked, or knotty. He observed that the hairs were normal root hairs, in that they were simple protuberances of single cells, although not strictly epidermal, but on or near the surface of the tissue. Yarbrough showed that the roots of Arachis have no intact epidermis, but have in its place a sloughing layer of outer cortex. It is interesting to note that he observed that hair formation occurred in an area where sloughing of the superficial layers is at a minimum. From his experiments, Yarbrough assumed that in the loose soils of peanut fields some hairs may be produced, even though difficult to demonstrate. No root hairs were observed in this study either on the embryos germinated in the laboratory or on the plants grown in the greenhouse and no concentrated effort was made to demonstrate their presence.

Reed (1924) presented a paper of anatomical interest that was strongly oriented along the lines of an embryological study of Arachis hypogaea. In the publication, Reed described the structure of leaf, stem, root, and flower. These descriptions, though brief, are apparently quite accurate. He very briefly describes the seed and seedlings of the plant. Reed also gave a written description of the embryo sac and development of the embryo, supported by explanatory diagrams. The embryological description and diagrams are concise, but accurate, and

are of a more useful nature than those of previous references. In addition to the above material, he describes the anatomy of the gynophore briefly and provides some rather diagrammatic representations of the gynophore and its vascular bundles. This portion of the work is interesting from the anatomical viewpoint and is generally accurate. His diagrams of the gynophore bundles at the various stages of cambial development are accurate and serve to point out that the cambium does extend in a ring from bundle to bundle as interfasicular cambium. This is opposed to the view of Pettit (1895) and in agreement with those of Waldron (1919).

Two papers that would probably prove useful in an analysis of the anatomical work done with Arachis hypogaea are the works of Thompson (1929) and Russell (1931). These papers are known only through the work of Smith (1950). Smith states that "Thompson and Russell have described the anatomy of the fruit during development and at maturity". Smith goes further to explain the structures of the pericarp as described by Thompson and Russell. The description is quite accurate, but nothing can be said concerning the completeness of the works, since they have not been seen by the author and are not available in the V.P.I. Library.

An excellent paper concerning the development of the gynophore of Arachis hypogaea was published by Jacobs (1947). In his work Jacobs determined the extent of the intercalary meristem responsible for the elongation of the gynophore. This was accomplished by mapping the distribution of the mitoses seen in longitudinal serial sections of the gynophore. The work contains only a limited number of photomicrographs

useful in an anatomical study, but an excellent written description of the gynophore anatomy is included. A concise and helpful description of the methods used is also included. Jacobs observed that xylem elements and phloem elements traversed the length of the gynophore intercalary meristem, thus casting doubt upon the theory that delay in fructification or ovarian development might result from insufficient movement of nutrients across the elongating meristem. A correlation between the absence of pitted or reticulate xylem elements and the occurrence of rapid elongation was noted. The evidence in favor of the fact that the first formed xylem elements undergo a great deal of stretching in the region of elongation is quite strong. Equally strong is the evidence favoring the idea that the separation of the helical turns or of the annuli of xylem elements is due to stretching.

Without question the best single reference seen concerning the anatomy of Arachis hypogaea is a paper by Yarbrough (1949). In this work, he describes the seedling, cotyledons, hypocotyl, and roots of Arachis hypogaea from an anatomical viewpoint. His paper contains useful written descriptions, and a helpful tabulation of the methods used in the study. It is interesting to note that the Virginia Bunch variant was used in his studies, whereas this author used the Virginia Runner variant. Yarbrough described the maturation of tissues in the primary root from studies of the root apex and absorptive zone as well as the developing vascular cylinder. The anatomy of the transition zone is clearly described in diagrams and written descriptions. He briefly described the changes that take place in the cotyledons during germination

and growth, but did not give a detailed description of the vascular system that develops in the cotyledons. He noted that the metaxylem of the peanut root visibly differentiates before the protoxylem, although wall lignification actually occurs first in the protoxylem. From these observations, Yarbrough concludes that justification for determining protoxylem and metaxylem should be based upon the speed or completion of maturation rather than upon relative time of initiation of differentiation.

Yarbrough (1949) made reference to two papers that have not been seen by this author and are probably of value in an analysis of the anatomical studies of Arachis hypogaea. According to Yarbrough, Badami (1935) provided descriptions of mature leaf, stem, and root anatomy, but the studies were considered to be of little use in his study of seedling development. As has been indicated, Badami also was a worker who observed root hairs on the plant. Yarbrough refers to Compton (1912) as being interested in phylogenetic principles and states that his work was published in the form of a broad comparative study of seedlings of the Leguminosae. According to Yarbrough, Compton was concerned mainly with the vascular structure of the Arachis seedling. Yarbrough noted numerous contradictions between his work and that of Compton. He thought that this might be due to the fact that Compton used a different variety than that of his own work, although Compton did not mention the variety studied. However, when Yarbrough made comparative studies on seedlings of Spanish and Valencia varieties, no significant variations from the Virginia type was observed.

An excellent review of the literature pertaining to Arachis hypogaea and the most complete bibliography yet seen concerning the plant was presented by Smith (1950). A summary of the widely dispersed, often contradictory, and in some cases obscure literature was brought forth and a valuable attempt to correct persisting errors in the literature was made by Smith. He provided an excellent description of the external morphology of the flower and the subsequent development of the fruit. Numerous explanatory diagrams are used to describe flower parts and to explain the method of transition between aerial flower and subterranean fruit. Smith proposes the use of the term "peg" in botanical literature in reference to the stalk-like stages in the development of the fruit that have been referred to as gynophores by other authors. He states "...a gynophore is usually defined as a stalk or stipe which supports the ovary or gynoecium in certain flowers...". He further states that the term is not appropriate when applied to Arachis, for the stalk is not distinct from the fruit proper, but arises from the elongation of the ovary itself. The term gynophore is used in this thesis consistently in order to avoid the confusion of synonymous terms in the literature, although the term peg may be more appropriate as a descriptive term. Even though the stalk-like structure arises from the base of the ovary, the structure is seldom considered a portion of the mature fruit. In this sense, even though not entirely correct, the gynophore does represent a separate entity from the mature fruit.

III

THE INVESTIGATION

A. Materials

The plant materials utilized in this investigation were various parts of plants of Arachis hypogaea f. macrocarpa and consisted of: (1) vegetative parts of growing plants, (2) flowers at varying developmental stages from growing plants, (3) developing gynophores from growing plants, (4) mature field-dried peanut fruits, and (5) parts of germinating seeds at varying stages of development.

A collection of vegetative parts of growing plants was made from plants grown in the V.P.I. greenhouse. Seeds for the plantings were obtained from the Tidewater Field Station. The pericarps were removed from the seeds and the seeds were shaken in a jar with a small amount of Aerasan to retard fungal growth. The seeds were soaked in tap water for twenty-one hours and placed in a moist chamber at room temperature. After a period of three days, the germinating seeds were planted in steamed soil at the V.P.I. greenhouse in a room that was maintained at an average temperature of 75° F. At the end of 39 days the first collection of material was made from the plants. At this stage the central upright branch had reached a height of 7-8 centimeters from the surface of the soil to the tip of the terminal bud. In the collection, plants were removed from the soil and the parts were cut from them and placed immediately in Formalin-Acetic Acid-Alcohol killing and fixing solution (hereinafter referred to as FAA and described in detail in

the section on Methods), where they were allowed to remain until utilized. The parts collected and used in this study were leaflets, petioles, stems, roots, transition zone sections, and cotyledons.

Two collections of flowers were made from the plants grown in the V.P.I. greenhouse. The first collection was made seventy days after planting. In this collection, sections of stems at the nodes were removed. These nodes all bore flowers that had wilted and had not yet developed gynophores and some bore undeveloped flower buds. The second collection was made eighty-six days after planting and sections of stems at the nodes were again collected. Some of the nodes visibly bore both a withered flower and a flower in anthesis, some visibly bore only a flower in anthesis, and some visibly bore both a withered flower and a developing gynophore. The flowers were placed in Formalin-Propionic Acid-Alcohol killing and fixing solution (hereinafter referred to as FPA and described in detail in the section on Methods) immediately after removal from the plants and were left in the solution until removed for use in the investigation.

Developing gynophores were collected from plants grown outdoors in pots during the summer of 1954. The series of plants used in this collection were grown in Blacksburg, Virginia by the author from seeds supplied by the Tidewater Field Station. Gynophores at various stages of development were cut from the plants and placed immediately in FAA, where they were allowed to remain until utilized. Some of the gynophores were elongating and had not yet reached the ground when collected; others were elongating, had reached the ground, and were beginning to enlarge

at the tips; and others had stopped elongating after reaching the ground and their tips had enlarged to approximately one-half to two-thirds of the mature size of the average fruit.

Mature field dried peanuts were collected at the Tidewater Field Station in October 1951 and in October 1953 by members of the Field Station staff. The pericarps of the fruits in the 1951 collection were removed and the seeds and pericarps were placed in FAA. The samples were allowed to remain in the killing and fixing solution until utilized in the investigation. Mature fruits from the same lot of seeds as that used in the greenhouse plantings were also used to furnish material for the study of dormant seed parts when the use of material that had not been killed and fixed was desired.

Germinating seeds were collected at various developmental stages for study also. In this portion of the investigation seeds obtained from the Tidewater Field Station were treated with Aerasan in the same manner as those used in the greenhouse plantings. The seeds were soaked for seven hours in tap water and then placed in a moist chamber at room temperature. Germinating seeds were collected after four days and the cotyledons were removed from the remainder of the embryo and cotyledons and embryos were placed in FAA where they remained until utilized. The embryos at this stage were approximately one centimeter in length, measured from the tip of epicotyl to tip of hypocotyl. Collections were also made at the end of seven days and nine days and were treated in the same manner as the collection at four days. At seven days the

embryos were approximately 1.5 to 2 centimeters long and at nine days they were 2 to 3 centimeters long.

B. Methods

The methods utilized in this investigation may be listed and described under the major headings of (1) Killing and Fixation, (2) Preparation of Material for Microscopic Examination, and (3) Observation and Description of Prepared Slides. Throughout the investigation constant reference was made to Johansen (1940) for information concerning microtechnique involved in the study.

1. Killing and Fixation

The killing and fixing fluids utilized for the various plant parts in this investigation have been mentioned previously. As has been explained, Formalin-Acetic Acid-Alcohol killing and fixing solution (FAA) was used for the vegetative parts of growing plants, developing gynophores from growing plants, mature field-dried peanut fruits, and parts of germinating seeds. Formalin-Propionic Acid-Alcohol killing and fixing solution (FPA) was used for the flowering material.

The FAA was prepared by mixing formalin (40% formaldehyde in water), glacial acetic acid, and 70% ethyl alcohol in the proportions of 5 to 5 to 90 respectively. The FPA was prepared by mixing formalin, propionic acid, and 70% ethyl alcohol in the proportions of 5 to 5 to 90 respectively. Materials placed in both FAA and FPA were allowed to remain there for at least 24 hours to insure proper fixation. It is felt that at least 96 hours should be allowed for the killing and fixation of mature whole fruits, although they remained in the FAA much

longer in this study. The practice of removing the pericarp from the fruit before placing in the solution is also to be recommended. Materials killed and fixed in both solutions were allowed to remain in the solutions until they were used for further study. Materials that contained air and floated when placed in the killing and fixing solutions were placed in jars approximately one-half full of the solutions and the air was evacuated with a faucet aspirator and the material was allowed to remain in the solution under decreased air pressure for approximately twelve hours. Leaves and flowers were constantly treated in this manner.

2. Preparation of Material for Microscopic Examination

The preparation of the material for microscopic examination as discussed in this thesis consists of three phases (1) preparation of material for mounting on slides, (2) mounting on slides, and (3) staining of material. Three different general techniques were used to prepare the material for mounting on slides. These were, (1) maceration of killed and fixed material, (2) sectioning of unembedded dormant seed material with a sliding microtome, and (3) serial sectioning of killed and fixed material, embedded in paraffin, with a rotary microtome. The staining techniques used consisted mainly of a stain of safranin only, a safranin and fast green combination, and a quadruple combination of safranin, fast green, crystal violet, and orange G. Other stains used to determine the constituents of certain cells were iodine-potassium iodide, chloro-zinc iodine, Sudan III, and Sudan IV. All of the crystalline stains were certified by the Biological Stain Commission.

The maceration technique of Jeffrey as outlined by Johansen (1940) was used in this study with several modifications. The only parts that were macerated were the pericarp and the seed coat. This material was obtained from the mature fruits in FAA from the 1951 and 1953 collections. It was used directly from the FAA without washing or dehydration. Before maceration the pericarp was dissected into: (1) the epicarp, (2) the vascular bundles, (3) the fibrous mesocarp, and (4) the endocarp. These portions were cut into small sections, as was the seed coat, and repeatedly boiled and cooled in distilled water to remove the air from the structures. To avoid the use of a macerating fluid too concentrated for the tissues, diluted mixtures of Jeffrey's fluid were used. One-tenth aqueous solutions of Jeffrey's fluid were mixed with the various components of the pericarp and the seed coat in vials at a temperature of 37° C. for 17 hours. Since this concentration was not rapid enough in its action, it was replaced with a one-fifth aqueous solution of Jeffrey's fluid for 24 hours at 39° C. This solution also proved unsatisfactory and a one-third aqueous solution of the maceration fluid was used to replace it. In this strength the temperature was held at 42° C. for 5 hours and then raised to 55° C. for 24 hours. The macerated tissues were then washed thoroughly in three changes of distilled water. In later experiments it was found that one-fifth strength Jeffrey's fluid for 17 hours at 60° C. was sufficient to macerate the pericarp bundles, but one-third strength maceration fluid at 60° C. for 17 hours plus an additional four hours in one-half strength maceration fluid at 60° C. was required to macerate the fibrous mesocarp.

The only materials sectioned on the sliding microtome were cotyledons from dormant seeds of the same lot of seed peanuts used for germination in the laboratory and plantings in the greenhouse. These cotyledons were sectioned without embedding at forty microns thickness. It was necessary to use such a great thickness, because thinner sections were difficult to handle unembedded and because the constituents of the cells were lost too readily. It was important to retain the cell constituents if possible, as will be shown below.

By far the largest part of the material prepared for microscopic examination was sectioned serially after embedding in paraffin. The types of materials handled in this manner were: whole peanut fruits, whole peanut seeds with seed coats, whole peanut seeds without seed coats, parts of mature fruits and seeds, gynophores and young developing fruits, leaflets and petioles, stems, roots, flowers, and germinating seed parts. Various dehydration, infiltration, and embedding schedules were experimented with during the early portions of the study. The schedule used for mature fruits, seeds, parts of fruits, and seeds, immature fruits and gynophores, leaflets, petioles, stems, roots, transition zones, and cotyledons from mature plants is shown in Table 1 Column A. The schedule used for flowers and parts of germinating seeds is shown in Table 1 Column B. The times of retention of tissues in the different solutions appears to be quite long and possibly proper dehydration and infiltration could be accomplished with much shorter periods of time. During the earlier stages of the study, attempts were made to properly embed complete fruits and complete seeds. Even with periods of

ninety-six hours, it was found that it was necessary to puncture several small holes in the pericarps and seed coats of these parts to properly prepare them for sectioning. Since these structures required the long periods of time, the practice of subjecting all parts to the same times was used. Due to the fact that satisfactory results were obtained with the schedules, no experiments with shortened times were attempted. During the dehydration if any structures were observed to float, the air was partially evacuated from the jars containing the structures and dehydrating solution in order to remove the excess air from the structures. The teriaty butyl alcohol method of dehydration and infiltration as outlined by Johansen (1940) was used with the modifications in time mentioned above. The solutions shown in Table 1 were prepared according to the directions for the preparation of the solutions in a tertiary butyl alcohol dehydration series as given by Johansen (1940). As suggested by Johansen, the plant parts were not washed upon removal from the FAA or FPA, but were placed directly in the 50% alcohol stage of the dehydration series. Fisher Tissuemat was used to embed all plant parts and sectioning of the embedded material was accomplished with a rotary microtome. Sections were cut at 10 to 20 microns thickness. The sectioning of complete fruits by this method was abandoned after numerous attempts, due to the difficulties experienced in infiltration, sectioning, and mounting on slides. It was found expedient to handle the parts of mature fruits separately.

Haupt's adhesive was used to mount the macerated tissues and paraffin sections on slides prior to staining. The freehand sections of

dormant cotyledons were first stained and then mounted directly in water. The Haupt's adhesive was prepared and used according to the instructions given by Johansen (1940). The Haupt's adhesive was not found to be satisfactory when used to affix pericarp and seed coat sections to slides. A 1% to 3% solution of celloidin in 50% ethyl ether - 50% absolute ethyl alcohol was added by pipette to the surfaces of slides upon which the pericarp and seed coat sections had been mounted with Haupt's adhesive. The paraffin had first been removed from the sections by immersing carefully for 1 to 2 minutes in a petri dish containing xylene. The celloidin solution was allowed to solidify and the solidified layer of celloidin was used to affix the sections to the slides throughout the staining schedule. The celloidin was removed by placing the slides in a petri dish containing 50% ether - 50% absolute alcohol for fifteen minutes, after the second change of absolute ethyl alcohol during dehydration in the staining procedure (See Table 3).

Several staining schedules were used experimentally during the course of this study. The methods shown in Tables 2, 3, and 4 gave the most satisfactory results and were finally utilized constantly. The quadruple combination of Conant (Johansen 1940) was used with minor modifications for all paraffin sections studied except for the pericarp and seed coat sections and is outlined in Table 2. The pericarp and seed coat sections were stained with a safranin-fast green combination (Table 3). Macerated tissues were stained with safranin only (Table 4). The tables list the solutions used and the times involved in the procedures. The cotyledons from dormant seeds that were sectioned on the

sliding microtome were handled as freehand sections and several staining techniques were used with them to determine the nature of cell contents. The staining techniques utilized consisted of a 2% aqueous solution of iodine potassium iodide for 1 minute, a chlor-zinciodine solution (Youngken 1930) for 10 minutes, a saturated solution of Sudan III in 95% ethyl alcohol for 10 minutes, and a saturated solution of Sudan IV in 95% ethyl alcohol for 10 minutes. In the use of each of these stains the sections were not taken through a dehydration series, but were placed in water immediately after sectioning and from this water immediately into the stain solution. The sections were rinsed in water upon removal from the stain for one minute and then mounted in water for immediate study.

3. Observation and Description of Prepared Slides

The finished slides were carefully studied microscopically with different powers of magnification ranging from 20X to 970X. Measurements were made with an ocular micrometer that had been previously calibrated. Diagrams of cell types were made with a camera lucida, photomicrographs were taken with a Leitz "Makam" camera, and copy work was done with a Crown 4 x 5 inches camera. The written descriptions produced as a result of this study are included in this thesis as "Results" and reproductions of the plates and photomicrographs are located at the end of the paper.

C. Results

The results of this investigation are here presented in the form of written descriptions with reference being made to diagrams and photomicrographs of various structures. The anatomy of the plant has been studied and is described under the basic sub-headings of (1) The Root, (2) The Stem, (3) The Leaf, (4) The Flower, (5) The Elongating Gynophore, (6) The Developing Fruit, and (7) The Mature Fruit. The transition region is not described as such, although reference is made to the zone in the descriptions of the root and stem. Yarbrough (1949) has presented a rather thorough description of this region of the plant.

1. The Root (See Plate 1 and Photomicrographs 1 and 2)

In this investigation cross-sections of the primary root at varying levels and cross-sections of the lateral roots were studied. Longitudinal sections through the transition zone were utilized to some extent in the study also. However, no work with the root tip and primordial tissues or with longitudinal sections of the root system at depth was performed in the investigation.

A cross-section of the primary root at the level of the highest lateral roots showed the epidermis to be broken down and sloughing off. Sections at a greater depth showed the epidermis to be lacking and as has been described by other authors (Waldron 1919, Yarbrough 1949), the outer surface consists of sloughing cortical layers. The cells of the cortex in the region of the first lateral roots were observed to be large parenchymatous cells varying in size from approximately 50 microns to

slightly over 100 microns in diameter. The cells of the cortex are polyhedral in cross-section with numerous intercellular spaces that are triangular in cross-section. The polyhedral shape of the cells has been more or less obliterated by the crushing of the cells that has caused infolding of the walls and breaking down of the cells. The smallest cortical cells are those toward the outside of the root. The cells are largest near the center of the cortex and those toward the inside are almost as small as those to the outside. There are intergradations in size in the regions separating the three layers. Irregular pit fields containing numerous small round to oval shaped simple pits were observed on the transverse walls. Pits were also seen on the radial and tangential walls of cortical cells in a longitudinal section of the transition region at a point approximately one centimeter below the point of attachment of the cotyledons. The pits on these walls are much larger than those of the transverse walls, are elongate-elliptic in shape, and are quite numerous. Nuclei are present in some cells at least and no stored food or other inclusions were noted in the cortical cells. At greater depths the cortical cells are essentially the same, although apparently smaller in size, and show a more flattened and infolded condition with nuclei observed less frequently. The cortex of lateral roots observed in this study is practically the same as that of the primary root. Here the cells range in size from approximately 20 microns to 35 microns in diameter. The inner 4 or 5 cell layers of the lateral root cortex show some areas of cells breaking down and the remaining cells exhibiting infolded walls due to crushing. The layers of cortical cells

to the outside of these intact 4 or 5 layers were represented by strongly flattened and crushed cellular material.

Yarbrough (1949) reported the existence of an endodermis in the root of Arachis as the innermost layer of the cortex. He identified this layer by its possession of Casparian strips. In a cross-section of the primary root at the level of the first lateral roots it was noted that the innermost cortical layer possessed uniformly thickened walls and was made up of cells smaller than the outer cortical cells. These cells are square to rectangular in cross-section with the majority of the cells of approximately 25 to 35 microns by 35 microns in size. The layer of cells is apparently endodermoid in nature, but the presence of Casparian strips could not be verified. All attempts to observe the presence of these stucture in this study gave negative results. The fact that a number of cell divisions occurs in this layer of cells is worthy of note. No numerical studies were made in connection with this phenomenon, although in the material observed it appeared that more radial divisions occurred than tangential or oblique, even though the latter two were also observed. Yarbrough (1949) suggested that the endodermis might be instrumental in adding cells to the sloughing cortex. Apparently the data indicate that this might occur. If the observation that the greatest number of divisions are radial is valid, the multiplication of the cells would evidently add most significantly to an expansion of the endodermoid layer, although the contribution of cells to the cortex might still be expected to occur. Sections of the primary root at greater depths showed the layer also, but some sections of the primary root indicate

that the endodermoid layer is sometimes crushed along with the cortex.

An endodermoid layer was also noted in lateral roots. Apparently this layer also consists of the innermost cortical cells. The cells were seen to be uniformly thick walled with nuclei. No actively dividing cells were observed in this layer of cells. The size of these cells is approximately 25 microns in tangential diameter by approximately 9 by 12 microns radially.

A pericycle is present in both the primary root and in lateral roots. The pericycle exists as a single or double layer of cells above the bundles of phloem fibers, sometimes widening to 4 or 5 layers between the patches of phloem fibers. In the material utilized in this study the pericycle was seen to be the tissue responsible for the development of a cork. Typical cork cells in 3 or 4 brick-like tiers were observed under the endodermoid layer and separated from it in some sections by an area in which the cork cells had broken down and separated from the adjacent endodermoid cells. Round to oval simple pits were noted on the transverse walls of the pericycle cells. Oval to elliptic pits are present on the radial and tangential walls of the pericycle cells as seen in a longitudinal section approximately 1 centimeter below the point of attachment of cotyledons.

The vascular organization of the primary root shows a radial arrangement of tissues. The xylem is tetrarch and the relative size of the tracheary elements indicates that development is centripetal. A section of the primary root at the level of the first lateral roots

showed the development of the primary xylem to have been exarch. The tetrarch pattern in the material used in this study was to some degree masked by the development of secondary tissues. The pattern can be determined most easily by observation of the four broad areas of phloem fibers and the four points of origin of lateral roots. Careful observation of the cross-section at the level of the highest lateral roots reveals four single rows of primary tracheary elements. The smaller and somewhat crushed elements (protoxylem) are seen to be to the epidermal side of the row, indicating exarch development. The cross-sectional diameters of tracheary elements varies from approximately nine microns diameter in the protoxylem elements through approximately 45 by 70 microns in the secondary xylem. Yarbrough (1949) in his developmental studies of the hypocotyl and root system of the plant describes the primary xylem of the root elements as consisting of "small peripheral ones having spiral thickenings while those progressively centrad are larger and may show scalariform and reticulate thickenings". He further states that both tracheids and vessels are represented in the xylem of the root. In this study it was observed that the smaller xylem elements in the stem and transition zone have spiral thickenings while the large ones possessed scalariform and reticulate thickenings. Some parenchymatous cells and fibers were also seen in the root xylem region.

At the level of the highest lateral roots the phloem fibers form four broad areas that cap the phloem areas and are 1 to 5 cell layers thick. Apparently the fibers in the material studied had not reached maturity at the time of collection as evidenced by the fact that in

almost all fibers the secondary walls appear to be detached from the primary walls and many secondary walls appear to be infolded. The lumina in many of the fibers at this stage are not as small as is often observed in mature phloem fibers. The fibers possess very small simple pits. In the material used in this study it was difficult to distinguish primary from secondary phloem. The metaphloem seive elements, companion cells, and parenchyma cells seem to merge with the secondary xylem with no line of demarcation. The phloem is seen to consist of about 10 to 12 layers of cells between the cap of phloem fibers and the cambium. It is made up of large parenchymatous cells and small bundles of 2 to 6 sieve elements and in some cases companion cells. Crushed protophloem elements were observed in the material to the outside of the areas of phloem fibers and just under the pericycle.

The interfascicular cambium gives rise to xylem and phloem elements and in a mature root the vascular tissues may be seen to be a continuous ring.

In cross-sections of germinating seeds, the cambium is seen to be active before maturation of the primary tissues has proceeded to any appreciable extent. Apparently some secondary elements mature prior to the completion of maturation of the primary elements. It was also noted that the development of lateral roots is initiated before the maturation of primary tissues is very far along. The lateral roots arise from the pericycle at the points where there are no primary phloem patches and their associated fiber caps. These regions are obviously the positions juxtaposed to the primary xylem bundles.

A rather extensive pith is seen in the primary root that is continuous with the pith of the transition region and the stem. The pith cells appear as large polyhedral cells in cross-section at the level of the first lateral roots. Numerous large intercellular spaces appearing triangular in cross section may be seen throughout the pith. The cells of the pith range in size from approximately 25 microns in diameter near the xylem to 70 microns in the center of the root. Near the transition region and also to some extent for a distance below this region, the pith cells contain a large supply of stored food material. The pith of deepest portions of the primary root were observed to possess large rhombohedral crystals of non-staining material. Numerous irregular pit fields containing round to oval simple pits were noted on the transverse walls. The pits on the radial and tangential walls of the transition region at a point approximately one centimeter below the point of attachment of the cotyledons are larger than those of the transverse walls and are essentially round or oval simple pits. They are located in elongate (in the direction of the root axis) pit fields that most often consist of a single row of pits. As has been observed by Yarbrough (1949) for cortical and pith parenchyma, the pit fields apparently often are continuous from the wall of one cell to that of another.

The lateral roots possess a diarch arrangement of xylem and the relative size of the tracheary elements indicates that their development is exarch. The size of these elements ranges from four microns in the protoxylem to about 30 microns in the metaxylem. The phloem is essentially like that of the primary root, although in the material examined very

little cambial activity had occurred and the two patches of phloem that alternate radially with the xylem are mainly primary phloem and are only 4 or 5 cell layers thick. The fibers have almost completed maturation. There is no pith in the lateral roots and the xylem is seen as a solid central core.

2. The Stem (See Plate 2 and Photomicrographs 3 and 4)

Cross sections taken from a portion of the main stem at a point about two centimeters above the point of attachment of the cotyledons were studied in this portion of the investigation. Longitudinal sections through the transition zone to a point approximately one centimeter above the point of attachment of the cotyledons were also utilized in the following descriptions.

The gross morphological character of the stem was described by Pettit (1895) as being cylindrical and smooth at the base and becoming angular and hairy above. In the cross-sections of the stem at a point two centimeters above the cotyledonary attachments, the stem appears to be somewhat four angled. This condition is apparently due to the presence of four large vascular strands that are offset from the vascular ring at about ninety degrees from each other. As nearly as can be determined morphologically, the four large strands supply the lateral branches at higher points in the stem. The cross sections show none of the surface hairs and only a few of the epidermal crystals that were noted by Waldron (1919) and Reed (1924). However, a portion of the epidermis was carefully stripped from a young living stem of a plant from the greenhouse

plantings and examined microscopically. Numerous 3-celled hairs (two small basal cells and one elongate terminal cell) as described by Waldron (1919) were noted in this material. Numerous epidermal cells were also noted that contained a large single rhombohedral crystal.

The epidermis of the stem is made up of cells whose outer and inner tangential walls appear to be thickened. Apparently there is a thin cuticle present on the outer surface of the layer. The cross-sectional size of the epidermal cells in this portion of the stem is approximately 12 to 20 microns in tangential dimension by 17 microns in radial dimension. The longitudinal section of the transition region shows the epidermal cells to be elongated to approximately 30 to 90 microns in the direction of the axis of the stem. Numerous stomata were noted in the epidermis. The guard cells, which are considerably smaller than the other epidermal cells and possess prominent outer ledges, are approximately 7 microns in tangential diameter by 12 microns in radial diameter. The stomata observed in this material were seen to be oriented with their longitudinal axis parallel to the axis of the stem. The guard cells are approximately 35 microns in length. The stomatal openings of the material used in this study were approximately 3 to 4 microns wide by 9 microns long.

The cortex of the stem in the region studied was observed to consist of 6 to 8 layers of cells as was reported by Waldron (1919). In some sections 5 or 6 layers of smaller cells were noted under the epidermis and the extent of the cortex in these places was seen to be 10 to 11

cell layers in thickness. The cells are large, appearing to be round in cross-section with triangular intercellular spaces. Rather large nuclei (approximately 10 to 12 microns) with 1 or 2 prominent nucleoli may be seen in many of the cells. The first 2 or 3 cell layers under the epidermis are collenchymatous and vary in size from approximately 80 to 100 microns in diameter. The largest cortical cells are those near the center of the cortex. These are parenchymatous cells that vary in size from approximately 180 to 220 microns in diameter. The innermost cortical cells are parenchymatous and range in size from 70 to 100 microns in diameter. The cells near the center of the cortex appear to be in the process of being crushed, with some cells that present a flattened appearance and others that have folded radial walls. In longitudinal section the cortical cells appear to be somewhat elongate and range in size from approximately 70 microns in length just under the epidermis through approximately 45 to 100 microns near the center of the cortex to approximately 35 to 60 microns in the innermost layers.

The innermost layer of the cortex consists of cells that are shorter in axial length as seen in longitudinal section than the other cortical cells, and they range in size from approximately 18 to 35 microns long. These cells are somewhat elongate tangentially in cross section and their approximate size varies from 25 microns radially by 35 microns tangentially to 20 microns radially by 40 microns tangentially. Occasionally these cells may be seen to contain large irregular rhombohedral crystals of a non-staining material. It is interesting to note that

these crystal cells were observed to be oriented in vertical rows in longitudinal sections.

Numerous simple pits like those described in the cortex of the root are present in the cortex of the stem. As in the root, they are simple oval to elliptic pits, tending to be larger on the radial and tangential walls than on the transverse walls. Spherical chloroplasts are present throughout the cortex.

The primary vascular tissues are arranged in the typical herbaceous dictyostele manner and are seen as a ring of vascular strands separated by rays of parenchymatous cells 1 to 3 cell layers thick. A considerable amount of secondary tissue was present in the sections utilized in this portion of the investigation and this secondary growth is described below. As was mentioned above, the stem in the region examined (approximately two centimeters above the point of attachment of the cotyledons) is somewhat four-angled in cross-section, because of four large vascular bundles that are removed or offset slightly from the vascular cylinder.

Each bundle in the vascular cylinder is capped by a bundle of extra-xylary fibers to the outside. Yarbrough (1949) has noted that the first phloem elements to form in the root of Arachis hypogaea are sieve tube members that always have at least one wall contiguous with the pericycle. Material from germinating seeds used in this study tends to confirm this observation and it was noted in the mature root cross-sections that obliterated cellular material, presumably crushed proto-phloem elements, is to the outside of the fiber caps. Apparently the development of the phloem in the stem is different from that of the root

because it was noted that phloem differentiation is initiated within the provascular strands and to the inside of the yet undifferentiated fiber bundles. With these differing situations in mind it may be seen that the extraxylary fibers of the root may be referred to as "phloem fibers", while such a term is apparently not precise in its meaning when applied to the stem. It is difficult to reconcile the popular concept of phloem differentiation as occurring constantly centripetally with the fact that "phloem fibers", whose initials are definitely a part of the provascular strands, differentiate to the outside of the protophloem after the protophloem has already differentiated. A pericycle cannot be distinguished from the cortex in the stem and the term "pericycle fibers" seems to be less accurate than "phloem fibers". Possibly the term "perivascular fibers" could be more accurately applied in this type of situation. Esau (1953) has used the term in reference to "fibers located on the periphery of the vascular cylinder inside the innermost cortical layer but apparently not originating in the phloem". The fiber caps are 3 to 5 cell layers thick and the fibers cross-sectional diameters range in size from approximately 5 to 20 microns, and present the normal polyhedral appearance of non-isodiametric fibers in cross-section. Although not too much secondary wall material was present in the fibers of the sections studied, it was apparent that the secondary walls were separate from the primary walls and showed infoldings as in the root. Simple pits are present in the fibers.

The primary phloem patches are seen directly under the fiber caps and they consist of 5 or 6 layers of cells that are in the process of

being broken and crushed. As nearly as can be determined from this material, the primary phloem in its active state consists of sieve elements, companion cells, and parenchyma cells. Apparently the line of demarcation between primary and secondary phloem is more easily recognized in the stem than in the root. One criterion used in distinguishing between the two being the fact that the cells of the primary phloem are larger in cross section than those of the secondary phloem. Also, as has been noted, almost all the cells of the primary phloem are in the process of being crushed and broken. None of the secondary phloem is in the process of being crushed at this stage. The secondary phloem consists of about 5 to 8 layers of sieve elements, companion cells, and parenchyma cells.

A typical cambium may be seen separating the secondary phloem from the secondary xylem and secondary tissue development is so extensive that the older stems are actually quite woody. The primary body is that of a typical herbaceous dictyostele and the secondary vascular tissues have the form of a continuous cylinder. However, ray tissue, usually to the extent of one to three rows of parenchymatous cells, is often seen to have developed from the interfascicular cambium, especially to the outside of the cambium.

The xylem elements range in cross-sectional diameters from approximately 7 microns in the greatly stretched primary xylem elements to approximately 50 microns in the mature secondary xylem elements. A longitudinal section through a vascular bundle in which some secondary tissues have developed shows the tracheary element closest to the pith

to have annular secondary wall thickenings that are separated considerably. Progressively outward in the bundle may be seen 3 or 4 tracheary elements with helical thickenings that are progressively closer together as the proximity of the element to the cambium increases. The innermost elements appear to have undergone considerable stretching and compressing. Usually one vessel with scalariform secondary wall thickenings is seen between the elements with spiral thickenings and the elements to the outside with scalariform-reticulate thickenings and the ultimate pitted vessels. Due partially to the great length of the stretched primary xylem elements, and partially to the difficulty encountered in observing the true extent of their length because of obliteration, the length of these elements could not be determined with any degree of accuracy. They are much longer than the elements of the secondary xylem. The pitted vessels toward the outside of the bundle have transverse end walls and are approximately 140 to 170 microns in length and approximately 40 to 50 microns in diameter. To the inside of these may be seen scalariform-reticulate and pitted vessels with sloping end walls that are approximately 200-300 microns in length and about 25 to 35 microns wide. All of the vessel perforation plates noted in this study were observed to be simple. The immature secondary xylem elements were noted to have numerous simple pits.

A typical pith is seen in the stem. This tissue area consists of large parenchymatous cells that are circular or polyhedral in cross-section, with numerous intercellular spaces that are triangular in cross-section. The cross-sectional diameters vary from approximately

35 to 70 microns. Their lengths vary from approximately 35 to 60 microns. Numerous small simple pits are present on the transverse walls, and as in the root, elongate pit fields with one or two rows of large oval to elliptic pits are present on the radial and tangential walls. These pit fields also apparently extend from the wall of one cell to that of another cell. In the material used in this portion of the investigation, the pith was observed to be irregularly breaking down and most of the cells exhibited folded walls. Such a condition would obviously lead eventually to a semi-hollow condition in the stem, as has been described by Waldron (1919) and Reed (1924).

Esau (1953) mentions the presence of tannin sacs in members of the Leguminosae. Solereder (1908) also records the presence of tannin sacs in the Leguminosae. Pettit (1895) recorded the presence of these idioblasts in the gynophore of Arachis hypogaea and suggested that their proximity to the vascular tissues indicated some function related to vascular functions. One, two, or three parenchymatous pith cells directly under some vascular bundles assume the function of tannin sacs in the stem. These cells are seen in stained cross-sections to be filled with an homogeneous reddish-brown material. Often in the stem sections the tannin sacs were observed to contain granular stained material rather than the homogenous material. Nuclei were observed in some of these cells. In longitudinal sections the tannin sacs were seen to be arranged in rather long vertical rows of cells in the pith. In the longitudinal section about one centimeter above the cotyledonary attachment the tannin sacs were observed to be quite long and are apparently

the product of many pith cells whose end walls have broken down. The contents of these elements were observed to be of the granular nature described above and they somewhat resemble a laticifer containing granular food material. Below this point in the transition region at a point about one centimeter below the cotyledonary attachments the cells were seen to be much shorter and even though the end walls are sometimes broken down, the more or less homogeneous cell contents do not appear to fuse into one duct but retain the shape of the original cells. Xylem vessels were sometimes observed in cross-section to be filled with the tannin material. No tannin sacs were observed in the root cross-sections below the point of origin of the first lateral roots.

3. The Leaf (See Photomicrographs 5 and 6)

The gross morphology of the leaf of Arachis hypogaea has been described repeatedly by numerous authors. It is evenly pinnately compound of four oval-shaped leaflets with entire margins and netted venation. Each leaflet has a very short petiolule of 2 to 3 millimeters length that blends into the mid-rib of the leaflet. Numerous prominent veins branch pinnately from this midrib and they are oriented at an angle that slopes strongly toward the apex of the leaflet. Between these branch veins is seen the rather inconspicuous network of anastomosing veinlets.

The mature petiole is usually about two inches in length, with two prominent ridges on the upper or adaxial surface and a somewhat rounded abaxial surface. Two sharply pointed stipules, about 2 to 4 centimeters

in length, are seen at the base of the petiole and fused with it along one margin for about one-half of their length.

A cross-section of the petiole indicates that a continuous epidermis, similar to that of the stem, is present on the adaxial and abaxial surfaces. The first 1 to 3 layer of cells under the epidermis of the adaxial surface are collenchymatous cells. This collenchymatous cortex is about six layers thick in the region of juncture of abaxial and adaxial surfaces and is at least partially responsible for the two prominent ridges seen on the adaxial surface. Usually one layer of cells that somewhat resembles the collenchyma of the abaxial side of the petiole lies under the epidermis of the adaxial surface. The characteristic wall thickenings are not as obvious in this layer though, and possibly the material has not matured or the collenchymatous nature of the cells does not develop to the extent of that on the abaxial side. The typically one-layered area of cells is sometimes two to four cell layers in thickness where the cells are smaller in cross-sectional diameter.

Just under the collenchymatous or hypodermal layer of the adaxial side of the petiole are located 2 or 3 layers of chlorophyllous cells that are somewhat smaller cells than the cells to the outside and inside of them. They are approximately square to rectangular in shape and vary in size from approximately 12 microns by 12 microns to 17 microns by 35 microns. They resemble somewhat in shape and placement the palisade cells of the leaf. The layer of cells extends around the outside of the two vascular bundles closest to the adaxial surface and under the

collenchymatous ridges. The layer is obvious to a point about halfway between the two lateral vascular bundles on each side.

The vascular system of the petiole consists of five collateral bundles that appear to be quite like those of the stem. They form an arc that is interrupted by rays of large parenchymatous cells about six cell layers thick. The two bundles nearest the adaxial side of the petiole are smaller than the three nearest the abaxial side. The xylem is found on the adaxial side of the bundles. A cap of extraxylary fibers 5 to 6 cell layers thick is seen on the abaxial side of each bundle. Very little secondary wall thickening is apparent in the fibers at this stage and no separation of secondary and primary walls or infolded walls were noted. The fibers seem to be immature elements with secondary wall thickenings in some of the cells and protoplasts are still present. The primary phloem consists of sieve tube elements, companion cells, and phloem parenchyma cells and is 7 to 8 cell layers thick under the fiber caps. Some of the primary phloem cells exhibit broken walls, which may indicate that the crushing and obliteration of the tissue is about to occur. About 4 to 7 layers of secondary phloem cells (sieve elements, companion cells, and parenchyma cells) are seen to the inside of the primary phloem, and as in the stem, the cells are smaller than those of the primary phloem. An apparently active cambium is present in the bundles, although no interfascicular cambium was noted.

The xylem of the three larger bundles consists of 6 or 7 rows of 3 to 5 rather large tracheary elements; that of the smaller bundles consists of 4 or 5 rows of 2 or 3 tracheary elements of smaller size.

To the adaxial side of the xylem is seen an area of 3 to 5 cell layers of thick walled parenchyma cells. This area extends between the rows of tracheary elements for almost one-half of the depth of the xylem. It is difficult to determine from size of the diameters of the tracheary elements whether development is endarch or exarch. It is assumed that the development is endarch, as in the stem. It is seen that several tracheary elements of xylem are in the process of being crushed to the adaxial side of the bundle, as are some of the cells on the adaxial side of the cap of thick walled parenchyma cells.

The area within the ring of vascular tissue is composed of parenchymatous cells that are continuous with the parenchymatous cells of the rays. The cells are polyhedral as seen in cross section and vary in size from approximately 40 microns to almost 200 microns in diameter. Triangular intercellular spaces as seen in cross-section are numerous.

The leaflets are attached to the petiole by very short petiolules. Cross-sections of a petiolule indicate that it is similar in several respects to the petiole. Near the petiole three collateral vascular bundles similar to those of the petiole, except that they are smaller, may be seen in the shape of an arc with its concave side to the adaxial surface. An epidermis similar to that of the petiole covers the outer surface of the petiolule. A single layer of collenchymatous cells is located under the epidermis as in the petiole, although the walls do not exhibit as profound thickenings. The petiolule in cross-sectional shape resembles the petiole. The ridges on the adaxial surface are not as prominent as those of the petiole however, even though the two regions

collenchymatous cells just under the epidermis is five or six cell layers thick. As in the petiole, two or three layers of chlorophyllous parenchyma cells are seen primarily on the adaxial side of the petiole just under the collenchymatous cells. As in the petiole, the cells appear somewhat square or rectangular in cross-section.

During the course of passage through the petiolule, the three bundles of the petiolule coalesce toward the center. The three bundles are still distinct, however, and each bundle is surrounded by an area of 4 or 5 layers of sclerenchymatous cells. The cells have very thick walls with simple pits. Protoplasts are present in many of the cells. The phloem area appears to be quite reduced in size and blends into the fibrous sheaths almost imperceptibly. No cambial activity was noted in the bundles. Each of the sclerenchymatous sheaths is coalesced into a mass of thick-walled cells on the adaxial side of the group of bundles. The rather large central mass of sclerenchymatous tissue was observed to enclose a large central tannin sac. In this region the collenchymatous cells under the epidermis and chlorophyllous areas seen in the petiolule at a point close to the petiole are no longer present. The central area of a mass of vascular tissues and sclerenchymatous cells is surrounded by about 10 cell layers of parenchymatous cells and the epidermis. The parenchymatous cells possess numerous large simple pits.

Cross-sections of the leaflet indicate that the mid-rib is similar to the petiolule described above. However, the sclerenchymatous area surrounding the vascular tissue is not as extensive as that of the petiolule, although still quite pronounced. Three or four tannin sacs

may be seen to the adaxial side of the vascular tissues. An upper and lower epidermis is present on the blade of the leaflet. The cells have thickened outer and inner walls with a thin cuticle present on the outer walls. The cells of the upper epidermis vary in size from approximately 35 microns in width (parallel to the surface of the leaf) by 35 microns in depth (perpendicular to the surface of the leaf) to approximately 70 microns in width by 35 microns in depth. Those of the lower epidermis vary in size from approximately 15 microns in depth by 50 microns in width to approximately 20 microns in depth by 90 microns in width. The cells of the lower epidermis are notably smaller in the region of the mid-rib and in cross-section these cells are approximately 15 microns in depth by 20 microns in width. Reed (1924) observed upper epidermal cells to project inward quite regularly, forming an inverted cone-shaped tannin cell. No such cells were observed in the material used in this investigation. Stomata were observed on both the upper and lower epidermis. These are essentially even with the surface, and quite similar to those seen in the stem. The guard cells are approximately 7 microns by 9 microns in cross-sectional size.

The palisade mesophyll consists of 3 or 4 layers of elongate parenchymatous cells. These cells contain nuclei and numerous peripheral chloroplasts. They are longest in the first layer under the epidermis and are shorter progressively toward the lower epidermis. The palisade mesophyll merges indefinitely into the spongy mesophyll, a loosely organized region below the palisade area that consists of small chlorophyllous parenchyma cells of irregular shape and placement. A layer of

quite large parenchymatous cells, apparently devoid of chloroplasts and cell inclusions, is seen between the spongy mesophyll and the lower epidermis. The cells range in size from approximately 70 to 100 microns in diameter in the blade, and are considerably smaller (approximately 30 to 60 microns in diameter) in the region of the midrib where the tissue is 2 to 4 cell layers thick. Waldron (1919) and Reed (1924) refer to this layer as water storage tissue. The layer of large parenchymatous cells does not extend to the lateral extremities of the blade. A densely packed bundle of small achlorophyllous parenchyma cells that are approximately nine microns in cross-sectional diameter may be noted in the tips between the epidermis and a vascular bundle. The palisade tissue is seen to extend to the lateral edges of the blade under the epidermis and above this group of small parenchymatous cells. The upper and lower epidermis is continuous around the lateral edges of the leaflets.

Vascular bundles of various sizes may be seen in a cross-section of the leaf. Some of the smaller bundles were seen to contain only two or three xylem tracheary elements. Each vascular bundle is surrounded by a sheath of at least one layer of achlorophyllous parenchyma cells that extends from each bundle, except some of the smallest, to the upper epidermis by one or two rows of cells similar to the sheath cells. The sheath cells are in contact with the layer of water storage cells below the bundles. It is interesting to note that non-staining rhombohedral crystals were often seen in the upper epidermal cells above the bundle sheath extensions. Sometimes the epidermal cells were seen to be absent

above these extensions, and in this case crystals were often seen on the surface, possibly the remainder of obliterated epidermal cells. Crystals were sometimes noted in the lower epidermal cells also. The bundle sheath cells may be seen in longitudinal section to be elongate in the direction of the vascular bundle. The mid-rib is surrounded by parenchymatous cells and an extension of parenchymatous cells to the upper surface of the leaf is four cell layers wide.

The mid-rib of the material used in this study consists of about 12 to 14 rows of 3 to 5 large xylem tracheary elements arranged in a fan-like pattern. To the adaxial side of the xylem is seen a group of 4 or 5 layers of immature sclerenchymatous cells that are similar to those of the petiolule and apparently continuous with them. Above this group of sclerenchymatous cells is seen a region of 3 or 4 layers of parenchymatous cells, of which 3 or 4 large centrally located cells have developed into tannin sacs similar to those present in the pith of the stem. Apparently no active cambium is present in the midrib. On the lower or abaxial side of the xylem in the mid-rib are seen the 8 or 9 layers of phloem cells consisting of 4 or 5 layers of small cells that are apparently sieve elements, companion cells, and phloem parenchyma, and 4 or 5 layers of immature fibers similar to those of the petiolule.

4. The Flower (See Plate 3 and Photomicrograph 7)

The morphology of the flower of Arachis hypogaea has been completely and accurately described by Smith (1950). Briefly, it is a papilionaceous flower that is perigynous, with a bilabiate calyx that is made up

of five fused sepals. The hypanthium is a very long tube enclosing the ovary and style. Borne upon the upper end of the hypanthium are the calyx, corolla, and androecium. The flowers are sessile in the axils of the leaves. The gynoecium consists of a single pistil with a club-shaped stigma, a very long style, and a small sessile unilocular ovary containing usually two ovules. The androecium is made up of ten monadelphous stamens. Of these, four have elongate anthers; three of which are biloculate; the remaining one uniloculate. These four stamens alternate with four others that possess globose uniloculate anthers. These eight stamens are of approximately the same length. The two stamens opposite the standard are sterile, having no anthers, and are usually of unequal lengths and shorter than the fertile stamens. The vascular system of the flower in the region of the ovules and the components of the ovary were the portions of the flower studied most intensively in the investigation because these portions are most important to an understanding of the structure of the fruit. Incidental observations concerning structures useful in the description of the flower vascular system and ovary are also recorded. The material used in the study consisted of serial cross-sections of a flower in bud and a flower in anthesis.

A cross-section of the flower in the region of the ovules shows the flower to be organized in two general areas; the hypanthium and the ovary. The hypanthium is essentially circular in cross-section. The outer layer of the structure consists of an epidermis with prominent nuclei and rather densely staining protoplasts. The features of the

epidermal cells are difficult to determine in the young cells of the flower in bud. The more mature cells on the epidermis of the flower in anthesis show thickened inner and outer walls.

Within the epidermis, the hypanthium is made up of 8 to 10 layers of parenchymatous cells. Apparently the structure has no epidermal layer on the inner surface, but the innermost layer of parenchymatous cells forms the inner surface of the hypanthium. The inner layer of parenchymatous cells is made up of smaller cells than the other parenchymatous cells in the hypanthium of the flower in bud. The inner layers of cells are seen in the flower in anthesis to be crushed and obliterated.

Embedded in the parenchymatous hypanthium of the flower in bud are twenty provascular strands. These strands are arranged in two rows, the outer row is situated two or three cell layers under the epidermis and the inner row has two cell layers between it and the inner surface of the hypanthium. In the flower in anthesis, as has been noted, these parenchymatous layers are seen to be crushed. Each provascular strand of the inner ring is oriented directly under a strand of the outer-most ring with three to six layers of parenchymatous cells separating them. Just under each of the outer provascular strands are one, two, or three (most often two) tannin sacs similar to those seen in other parts of the plant. These are filled with the characteristic reddish-brown homogeneous material in the stained sections of the flower in the bud. The provascular strands are apparently primordial collateral bundles with several protophloem elements that have matured at the outer extent of the bundles and from one to four xylem elements maturing to the inside of

the strands. The development of the xylem is apparently endarch in the flower parts. The inner ring of strands is similar to the outer, except that no tannin sacs are seen to the inside of the strands, and the strands are somewhat smaller than those of the outer ring. An interesting observation made during this investigation was the one concerning the size of the provascular strands of the inner rings. It may be seen that there are four strands that are larger (approximately 45 microns radial diameter by 75 microns tangential diameter) than the other six (approximately 35 microns radial diameter by 45 microns tangential diameter). Three of the smaller strands alternate with the larger strands, while three are in a series between two of the larger strands. By tracing these strands, in serial sections, it may be seen that the four largest strands supply the four stamens with elongate anthers, while the smaller strands supply the four stamens with globose anthers and the two sterile filaments. The provascular strands of the outer ring are of approximately the same size at this stage (approximately 70 microns radially by 90 microns tangentially). By tracing these strands into the upper flower in serial sections, it may be seen that five alternate bundles supply the calyx, while the other five supply the corolla. The cross-sections of the flower in anthesis show the vascular bundles to be fully developed. The variations in size of the inner ring of vascular bundles as described above is also apparent in the flower in anthesis. It is interesting to note that at this stage the contents of some of the tannin sacs associated with the outer ring

of provascular tissues exhibit the granular appearance described above for some tannin sacs of the plant axis.

The cross-sections of the flower in bud in the region of the ovules show the ovary within, and surrounded by, the hypanthium. There is no tissue connection between the hypanthium and ovary at this level. The ovary in this region is covered by an epidermal layer that is made up of rectangular shaped cells, as seen in cross section, with the long axes in the radial dimension. These cells range in size from approximately 5 microns tangentially by 9 microns radially to 10 microns tangentially by 12 microns radially. The cells have thickened outer walls and dense, darkly staining protoplasts. In the cross-sections of the flower in anthesis the ovary wall shows larger radially elongate epidermal cells than the flower in bud and the cells may be seen to possess thicker outer walls. In these sections the epidermal cells are approximately 8 microns in tangential diameter by 18 microns in radial diameter.

Inside the epidermal layer in the ovary of the flower in bud are seen 4 or 5 layers of parenchymatous cells surrounding a ring of smaller cells with large darkly staining nuclei. The ring of smaller cells is 3 to 5 cell layers thick and apparently nine segments of it are in the process of differentiation into provascular strands. These segments or provascular strands are 6 to 8 cell layers thick. One strand appears to be larger than the others and has several differentiated phloem elements, as well as 4 or 5 differentiated xylem elements. This largest strand may be traced up into the style in serial sections and is seen as the only provascular strand in the style. In the region of the

ovules of the flower in anthesis the wall of the ovary presents essentially the same picture as in the cross-sections of the flower in bud. In these sections apparently nine provascular strands are also present and most of them are in a state of active differentiation. Four or five xylem elements were seen to be differentiated in the largest bundle. Above the level of the ovules the style may be seen to be elliptical in cross-sectional shape with a canal that occupies one end of the ellipse and a provascular strand at the other end. The epidermis of the style of the flower in bud consists of somewhat cubical shaped cells with large nuclei and thickened outer walls. In the flower in anthesis the layer is not too easily differentiated from the ground tissue but it has a slightly thickened outer wall. The ground tissue consists of small parenchymatous cells with rather large nuclei and is three layers in depth between the epidermis and the canal and between the epidermis and the vascular bundle. There are two layers of this tissue separating the canal and the vascular tissue. The layer of cells lining the canal is apparently the innermost layer of the ground tissue and cannot be distinguished from the ground tissue in cross-section. The canal is continuous with the locule of the ovary. The style also has a ring of tannin sacs containing the characteristic reddish-brown material. The number of sacs varies with the level of the cross-section in the style. No tannin sacs were noted in the ovary wall while at one level in the style of the flower in the bud seven bundles were noted. In the flower in anthesis thirteen sacs were seen at one level. Progressively higher in the style the number is again reduced until none are seen. The sacs

are arranged in an arc that conforms generally to the shape of the epidermis of the style. The arc is oriented in about the third layer of parenchymatous cells under the epidermis and passes to the outside of the provascular strand. Tannin sacs in other parts of the plant have been seen only to the inside of the vascular tissue. The largest tannin sac is the one closest to the provascular strand, while those farther from the strand are progressively smaller. Where there were thirteen sacs noted, they were seen to extend in a circle completely around the style. Normally when less than this number is present, though, the arc is located mainly in the end of the ellipse in which the provascular strand is seen. Some of the smaller tannin sacs in the style of the flower in anthesis were observed to contain granular stained material rather than the homogeneous reddish-brown material of the larger sacs. The sacs in the style of the flower in anthesis all contain this granular stained material. The style of the flower in anthesis in cross-section appears quite similar to that of the flower in the bud, except that the cells are more mature.

To the inside of the ring of provascular strands in the ovary wall of both flowers are three or four layers of immature cells. The cells appear to be actively meristematic at this stage. The innermost layer of cells possesses notably large darkly staining nuclei.

In the ovules in both the flower in bud and the flower in anthesis, the integuments may be seen as six layers of cells. The position of the micropyle could not be determined from the slides used in this study. It was, however, fairly easy to determine which cell layers made up the

integuments by the presence of three cuticular layers in the ovules of the withered flower. The outer cuticle may be seen on the outside of the outer integument, which is 3 cell layers in thickness, except in the region of the funiculus where it was seen to be five layers thick. The median cuticle, between the two integuments is double in nature and shows the demarcation between integuments. The inner cuticle shows the point of separation of inner integument and nucellus. The inner integument is also three layers in thickness. The innermost layer of cells in this integument is made up of deeply staining cells with large nuclei. These cells are somewhat elongate radially as seen in cross-section and are believed to represent the integumentary tapetum (Esau 1953). A single layer of lightly staining cells that are apparently in a process of disintegration is seen lining the inner layer of the integument with a layer of disintegrated cell material within representing 1 or 2 layers. These layers of irregularly breaking down cells represent the nucellus. The funiculus appears to be continuous with the integumentary tissue and provascular tissue can be distinguished in the center of the structure. At this stage of development the provascular tissue of the funiculus connects with a provascular strand in the ovary wall at one end and disperses into the integumentary tissue without connection to a specific provascular tissue in the ovule.

The embryo sac of the flower in bud and that of the flower in anthesis may be seen in cross-section in the ovule surrounded by, and separate from, the disintegrating nucellus. The details of the structure of the embryo sac are masked by the presence of a large quantity of

stored food in the form of starch grains in the sac. This observation is in agreement with the observation of Reed (1924).

5. The Elongating Gynophore (See Plate 4 and Photomicrograph 8 and 9)

Smith (1950) has described the morphological nature of the development of the fruit of Arachis hypogaea. After fertilization the visible flower parts wither and usually drop off. Although no microscopic studies were made to determine the cause of the falling of the withered flower parts, it appears that the swelling ovary actually ruptures the hypanthium, thereby enabling the withered flower parts to drop off. In many cases the withered flower parts may be seen still clinging to the tip of the elongating gynophore. The style and stigma eventually wither and drop off, the ovary swells, and the base of the ovary develops meristematically, causing the appearance of the elongating gynophore. This developmental stage of the fruit is a stalk-like structure that is geotrophic in its elongation and that serves to carry the fertilized eggs, ovules, and ovary wall into the ground where further fruit development takes place. Jacobs (1947) has determined the meristematic region of the gynophore to be in an area located between 1 and 6 millimeters from the apex. This was accomplished by plotting the region of mitotic figures in the cells of the structure. Longitudinal sections of the apical five millimeters of an aerial gynophore and cross-sections of the apical region of a gynophore that had pierced the ground to the extent of about one-half to three-quarters of an inch were studied in this portion of the investigation. The major purpose of the study of this

phase of fruit development in Arachis hypogaea is to provide a link in the association of the various tissues and structures of the aerial ovary and the subterranean fruit.

The cross-sections of the apex of the gynophore show one vascular bundle to be present to one side of the structure and abruptly ending, as do the surrounding tissues, at the line of abscission of the style. The apex presents a somewhat hooked appearance, due to the unequal growth of the cells on the side opposite the one vascular bundle. The epidermis on this curved side of the apex consists of cells with heavily thickened outer walls and a very long radial dimension. This dimension was often observed to reach fifty microns, as compared to the thirty microns of the epidermal cells on the outer side of the gynophore. The lone bundle in the apex probably represents the one vascular trace of the fallen style. The canal of the style was not observed in the cross-sections. In the area of the ovules the epidermal layer resembles that of the ovary wall of the flower in that it consists of rectangular cells with thickened outer walls. These cells are approximately 12 microns by 30 microns, with the largest figure that of the radial dimension, as in the ovary wall. In the region of the funiculus of the ovule nearest the apex, about one-third of the circumference was noted to be without epidermis, as is the condition near the apex in which one-third to one-half of the circumference is without an epidermis. The reason for the absence of the epidermis in these areas is not known. The first three or four layers of parenchymatous cells under the epidermis contain large nuclei and have darkly staining cytoplasm. The cells of the first

and second layers under the epidermis, like the epidermal cells, appear in these sections to be radially elongate. There are no collenchymatous thickenings in these layers at this stage of development. Between the epidermis and the outer edge of the vascular bundles are five to seven layers of immature cells that are apparently still meristematic. The interfascicular areas consist of immature parenchymatous cells that are somewhat smaller than the cells of the cortex and the areas are about six cell layers in depth. No interfascicular cambial cells were noted at this stage of development. Just to the inside of the vascular bundles are eight or ten layers of immature cells that are evidently meristematic and that have large nuclei. These cells extend under, and blend imperceptibly with, the interfascicular areas. The cells of these inner layers are elongate tangentially and are quite immature as regards differentiation, as are all of the parenchymatous cells of this region of the old ovary wall, now the wall surrounding the locule of the gynophore. Apparently these cells still retain their meristematic properties.

In the region of the ovule nearest the apex of the gynophore, twelve or thirteen vascular bundles may be seen embedded in the tissue surrounding the locule. Many of the primary xylem and phloem elements are differentiated in each bundle and although a cambial layer appears to be present, it is not yet active. Tannin sacs containing the reddish-brown homogeneous material may be seen to the inside of many of the vascular strands.

The longitudinal sections of the gynophore show the tissues described above and present an overall picture of the apical five millimeters of the gynophore. Two ovules may be seen in a locule of the gynophore tip that generally conforms in shape to that of the two enlarged ovules. The radially elongate epidermal cells are plainly seen in these sections and have thick outer walls. They are approximately seven to twelve microns in the vertical dimension by approximately twenty-five microns radially. Three to five layers of vertically elongate parenchyma cells are seen under the epidermis. They range in size from approximately twenty-five microns to forty-five microns in the vertical dimension by ten to fifteen microns radially. This region of elongate cells blends into the region of smaller cubical to irregularly shaped inner cortical cells that contain large nuclei and darkly staining protoplasts. The four or five layers of cells lining the locule consist of very small cells with large nuclei. As was noted in the cross-sections, most of the cells are undifferentiated and appear to be meristematic at this stage of development.

The vascular bundles are seen to be continuous through the region of the ovules, as has been described by Jacobs (1947). The xylem in longitudinal section consists of tracheary elements with spiral thickenings. All of the perforations observed in this material were porous. The xylem is apparently endarch in its development.

Above the region of the ovules the center of the gynophore is filled with a pith that is made up of large parenchymatous cells that are

polyhedral in cross-section, with triangular intercellular spaces. The transverse walls possess irregular pit fields containing numerous oval-shaped simple pits. The cross-sections indicate that the pith cells are being crushed; a condition revealed by the infoldings in the cell walls. Jacobs (1947) has shown that the intercalary meristem of the gynophore is located in the pith at a level of 2.6 to 3.7 millimeters from the apex. For this reason the cells of the pith are very short in the vertical dimension in the region just above the locule and are progressively longer in higher regions of the longitudinal sections of the gynophore. In these higher regions, numerous large elongate-elliptic simple pits may be observed on the radial and tangential walls. In the outermost layers of the pith and just under the vascular bundles are seen the familiar tannin sacs. To a lesser extent these sacs are seen under the vascular bundles in the region of the locule. At this stage of development they contain the homogeneous reddish-brown material.

A cross-section in the region of the second ovule, or the one farthest from the apex of the gynophore, shows the tissues of the old ovary to be more highly developed. Within the vascular bundles the cambium is active and secondary xylem and phloem cells may be observed to be differentiating. The outermost cells in each bundle are differentiating into fibers. Provascular strands perpendicular to the longitudinal main bundles are developing between adjacent bundles and some mature vascular elements may be seen within these strands. A continuous ring of cells two or three cell layers thick, conspicuous because of its small cells, may be noted three or four cell layers below the innermost xylem elements

and encircling the much thickened area of meristematic cells lining the locule. Upon examination this continuous ring of two or three layers of cells is obviously made up of small developing sclerenchymatous cells. The area of immature parenchymatous and meristematic cells that lines the locule is now fifteen to thirty cell layers in thickness, as compared to the four or five cell layers seen in the region of the first ovule. The ring of vascular tissue in the region of the second ovule is composed of fifteen bundles. Obviously the second ovule and its surrounding tissue is more mature as a fruit than is the area of the first ovule. Reed (1924) and Smith (1950) have shown that the portion of the subterranean fruit to develop first is the region of the ovule farthest from the apex. On the basis of observations made on cross-sections of the gynophore in the region of the highest ovule, the young pericarp of the fruit may be broken down into the three usual areas. The epicarp at this stage of development is represented by the epidermis and the three or four cell layers below the epidermis that have densely stained cytoplasm and are collenchymatous. The mesocarp consists of the parenchymatous cells to the inside of the collenchymatous portion of the epicarp, the vascular bundles, and the developing sclerenchymatous ring of two or three cell layers within the ring of vascular bundles. The endocarp consists of the "parenchymatous-meristematic" cells within the ring of sclerenchymatous mesocarp and lining the locule.

The ovule nearest the apex of the gynophore has considerably developed, as seen in cross-section, since fertilization in the flower. The provascular strand of the funiculus linking the vascular system of

the pericarp with the integuments is more obvious, although at this stage of development no mature vascular elements may be noted. The outer integument is about ten cell layers thick at this stage of development and apparently still meristematic. The outer layer of this integument consists of square or rectangular cells in cross-section that constitute a developing epidermis. The inner integument is still made up of three cell layers with the inner layer (integumentary tapetum) still consisting of cells with darkly staining dense cytoplasm. The nucellus at this stage is represented only by a thin layer of crushed and almost obliterated cellular material lining the integumentary tapetum. The chamber of the ovule at this stage of development is lined by a layer of endosperm material, consisting mainly of large lightly staining nuclei, cytoplasmic material, and food material. The components of the layer are not separated into cellular components by cell walls, a condition described by Reed (1924). This endosperm is also present on the surface of the proembryo. The proembryo at this stage appears as a solid circle of cells in the center of the oval-shaped chamber of the ovule. The cross-sections of the proembryo show it to be about five cell layers in diameter. The ovule farthest from the apex in the cross-sectional slides appears to be essentially the same as the other ovule, except that it is slightly more developed.

The ovules are seen in longitudinal sections within the locule of the apical millimeter of the aerial gynophore. The exact position of the micropyle could not be determined in this material, but the placement of

the ovules at this stage of development indicates that the ovules are amphitropous, with the micropyle opening toward the apex of the gynophore (originally upward in the flower). The funiculi are attached laterally to the side wall of the locule. The provascular strand that links the vascular system of the gynophore with the integumentary tissue can be plainly seen and apparently no mature vascular elements are present. Both ovules are quite similar except that the one farthest from the apex of the gynophore is larger than the other. The proembryo is also larger in the ovule farthest from the apex. It is difficult to distinguish the integuments from each other in the longitudinal sections, but it appears that the inner integument is five cell layers thick near the micropylar end of the ovule and is two or three cell layers thick around the sides. A small area of nucellus is still present near the micropylar end of the ovules. It appears as a single layer of cells about five cells wide under the proembryo. The proembryo is two or three cell layers in width and about five cell layers in height in the ovule farthest from the apex. The proembryo of the ovule closest to the apex is two cell layers in width and about four cell layers in height. It is made up of smaller cells than the other proembryo and is noticeably smaller in overall size.

6. The Developing Fruit

A developing fruit was studied at this point in the investigation to observe the changes that occur in the apical portion of the gynophore of Arachis hypogaea during underground fructification. The material consisted of the subterranean tip of a gynophore that had begun to develop

into the familiar peanut fruit. The fruit at this stage was about three-quarters of an inch in length and one-quarter of an inch in diameter. Cross-sections were made through the region of the ovule farthest from the apex and longitudinal-sections were made through the region of the other ovule.

The epidermal layer seen on the elongating gynophore is not present on the developing fruit. Apparently this layer has been sloughed off. As seen in cross-section, the outer surface of the developing fruit consists of about seven or eight cell layers of badly crushed cells. Most of these cells still contain nuclei, but are probably in a state of being obliterated. As nearly as can be determined, they are parenchymatous cells. This would tend to indicate that the collenchymatous layers of cells as well as the epidermis have been sloughed off, leaving only the mesocarp and the endocarp as a remainder of the pericarp of the elongating gynophore. However, a layer of tissue is present on the outer surface of the mature fruit that has been called an epicarp by Winton (1904). Evidently the epicarp of the mature fruit is not derived from the epicarp of the elongating gynophore.

The outer parenchymatous region of the pericarp of the developing fruit varies in thickness from about fifteen cell layers over the vascular bundles to about thirty-five cell layers including the interfascicular areas. The region consists of the layers of the crushed cells already described plus a region of large nucleate parenchymatous cells that show signs of being crushed. The cells contain some stored food

in the form of starch grains, although the amount is far from copious. Numerous oval to elliptic simple pits of variable size may be seen on the transverse walls.

Bordering upon the innermost layer of the parenchymatous region of the mesocarp is the sclerenchymatous region of the mesocarp. This region, as in one portion of the elongating gynophore already described, consists of developing fibrous cells. This continuous ring of fibrous mesocarp is three to seven cell layers in depth in the developing fruit. Most of the developing fibers are evidently aligned with their long axes perpendicular to the plane of the cross-sections. However, many of the developing fibers are oriented with their long axes perpendicular to the axes of these, and a mass of interwoven fibrous cells is therefore being formed. This mass of sclerenchyma is in the shape of an irregular circle that consists of an undulating line of fibrous tissue forming alternate "crests" and "valleys". The "valleys" are broad arcs that have their concave surface oriented toward the outside of the young fruit. The "crests" are seen between the broad concave arcs and are segments of the sclerenchymatous ring that appear to be much narrower arcs, whose convex surfaces are oriented toward the outside. The "crests" are located under each vascular bundle and developing fibrous cells are seen projecting obliquely from the "crest" of the sclerenchymatous layer up on either side of each vascular bundle. In cross-section, then, a variable x-shaped pattern of fibers is seen beneath each bundle.

Thirteen vascular bundles were seen in the cross-sections of the developing pericarp in the region of the ovule farthest from the apex.

The vascular bundles are larger representatives of the vascular bundles of the old ovary wall that are more fully developed and contain more cells than were seen in these regions of the flower and elongating gynophore. A cambium may be seen to be active within the bundles and secondary xylem and phloem are seen to be maturing. A cap of fibers to the outside of each bundle is evidently in a process of maturing, although the walls are not thickened to any great extent at this stage and protoplasts are still present in many of the cells. No development of an interfascicular cambium was noted in the pericarp. The vascular bundles vary somewhat in size. A typical bundle is seen to consist of about five radiating rows of three to five xylem tracheary elements, with the protoxylem elements located to the inside of the bundle. The functional phloem consists of about six or seven cell layers of sieve tube cells, companion cells, and parenchyma to the outside of the xylem. Tannin sacs are noted less frequently in the developing fruit than in the stem, leaf, and flower, but where seen, they are located to the inside of the vascular bundles and to the outside of the fibrous ring. They contain the characteristic reddish-brown homogeneous material in stained sections.

To the inside of the ring of sclerenchymatous mesocarp, the endocarp is seen in the developing fruit. An extensive proliferation of cells has taken place in this area when compared with the endocarp of the elongating gynophore. In some areas this parenchymatous region is over forty cell layers in thickness, and is seldom less than thirty cell layers thick in the region of the ovule farthest from the apex of this material. The cells in this region are similar to the parenchyma cells

to the outside of the sclerenchymatous mesocarp, but they are generally larger than the parenchymatous mesocarp cells. The cells of the endocarp are large parenchymatous cells that have infolded walls characteristic of cells being crushed. The cells appear to have been polyhedral in cross-sectional shape and seem to be quite compact, having no intercellular spaces. The cross-sections show numerous irregular simple pits on the transverse walls. There is some stored food material in the cells, as was the case in the parenchymatous mesocarp.

In the longitudinal-sections of the region of the ovule nearest the apex of the developing fruit, the same tissue areas as were seen in the cross-sections may be seen. The radial and tangential walls of the parenchymatous mesocarp cells contain numerous large simple pits similar to those of the transverse walls. The size and shape of the parenchymatous mesocarp cells in longitudinal-section appears quite similar to the size and shape seen in cross-section. The longitudinal sections show vascular bundles oriented perpendicular to the plane of the sections. These are located at about the same level in the pericarp as are the major longitudinal bundles. They are smaller than the longitudinal bundles and apparently form a system of minor bundles that connects adjacent longitudinally oriented major vascular bundles. Except for size, these minor transverse bundles are similar in all respects to the major bundles, even to the flange of sclerenchymatous tissue that extends up and around the lower and lateral surfaces of the bundles from the sclerenchymatous mesocarp. The sclerenchymatous mesocarp appears in longitudinal-sections as it did in the cross-sections. The major longitudinal vascular bundles

in longitudinal-sections showed the xylem to consist of tracheary elements with spirally thickened walls. As is usually the case, the helices of the innermost elements were widely separated, while those farthest from the center were quite close together. The cells of the endocarp in the longitudinal-sections are quite similar in appearance to those seen in the cross-sections in all respects, including cell size and shape, as well as pitting, infolding walls, and food storage content.

The cross-sections of the ovule farthest from the apex show the ovule to be essentially similar to that of the elongating gynophore, except that the parts are in a more advanced state of development. An epidermal layer is seen as the outer layer of the ovule. The cells of this epidermis are approximately square in shape as seen in cross-section, with dense darkly staining protoplasts and large nuclei. The cell walls are not unusually thick at this stage of development. Under the epidermal layer, which is the outermost layer of the outer integument, the remainder of this expanded integumentary area may be seen. The outer integument, other than the epidermal layer, is seen to consist of about twenty-five cell layers of immature parenchymatous cells that are quite variable in size. Those cells just under the epidermis are the largest, while those nearest the center of the ovule are the smallest, with intergradations in size between the two extremes. The cells are nucleate and appear to be round in cross-section with triangular intercellular spaces. About the first ten cell layers under the epidermis exhibit infolding cell walls and a small amount of stored food material in the form of starch grains. The cells to the inside of this still possess the round

shape without infoldings of the walls and contain considerably more stored food material in proportion to their size than do the outer layers. Developing vascular bundles may be seen embedded in the outer integument at a level just to the outside of the region of the smaller parenchymatous cells that are so obviously high in food storage. Nine bundles were noted in the cross-sections in the region of the ovule farthest from the apex. The two bundles on either side of the funiculus were the most highly developed bundles and at this stage exhibited about twenty-five differentiated spiral tracheary elements to the inside of the bundle, arranged in somewhat of an irregular mass. The tracheary elements show very little variation in size and several elements were noted in the process of differentiation. About twenty differentiated phloem sieve elements, and more in the process of differentiation, were seen to the outside of the bundle, with no indication of a differentiating fiber cap at this stage of development. Apparently an active cambial layer is present in the bundles. The bundles on the side of the integument opposite the funiculus at this stage are merely provascular strands. One of the strands in this region contains one sieve element in the outermost layer of cells of the provascular strand. The vascular bundle of the funiculus appears to be quite well developed in these sections and is seen to connect the vascular system of the pericarp with the two bundles situated on either side of the funiculus in the outer integument. The tracheary elements of the bundle have spirally thickened walls. The inner integument in the cross-section is represented by two cell layers that seem to be in the process of obliteration. Evidently the integumentary

tapetum has been utilized by the developing embryo or endosperm. To the inside of the inner integument and lining the inside of the locule is the one to three cell layers thick endosperm; which is divided into cellular units by cross walls at this stage. The endosperm cells contain large nuclei and densely staining protoplasts, as compared to the inner integumentary cells with smaller nuclei and lightly staining protoplasts.

The proembryo at this stage of development consists of a mass of a large number of meristematic cells. The primordial cotyledons are quite obvious at this stage, although no cellular differentiation has yet occurred.

The longitudinal-sections of the ovule nearest the apex confirm the location and description of the cells and tissues as they were recorded from the cross-sections. The longitudinal-sections show the xylem tracheary elements in the outer integument to have spiral wall thickenings. The proembryo in longitudinal section appears in the form of the letter Y. The basal stem of the Y is represented by the suspensor and developing hypocotyl, while the two branching lines at the top of the basal stem are represented by the developing cotyledons.

7. The Mature Fruit

(See Plate 5 and Photomicrographs 10 through 14)

The mature fruit or matured pericarp with fully developed seeds was studied in order to determine its anatomical nature. In this portion of the investigation, cross-sections and longitudinal-sections of the pericarp, seed coat, and embryo with cotyledons, macerated tissue from

the pericarp and seed coat, and cross-sections of germinating seeds were studied.

The pericarp is the familiar "husk" or "shell" of the peanut. A cross-section of the structure reveals the same general tissue areas as those seen in the cross-sections of the developing fruit. The outer region consists of the more or less crushed remnants of the parenchymatous mesocarp of the elongating gynophore. The sections used in this portion of the study did not exhibit the epidermis or epidermal hairs reported by Winton (1904). The outer five to seven cell layers that encircle the fruit in cross section are seen to consist of three different types of cells. These three types of cells seem to be in more or less distinct layers. The outermost layer is composed of two or three layers of crushed and in many cases obliterated cells and cellular material. These cells are arranged in tiers. The one to three cell layers under these cells are made up of thick-walled rectangular-shaped cells that retain safranin in a safranin and fast green stain. These cells possess numerous large irregular simple pits. Winton (1904) has described the cells of what he terms the "hypodermis" in a manner that seems to indicate that it is this layer that he is describing. These cells are compactly arranged with no intercellular spaces. Where two or three cell layers are present, these cells also appear to be arranged in tiers. The slides made from macerations of the outer layers of the pericarp show the cells from this tissue with their numerous simple pits on all cell walls. As has been described, they are approximately rectangular in cross section. The macerated material shows the cells to be essentially

quadrilateral in surface view. To the inside of these layers are about three to five layers of cells that have the same type of irregular large simple pits and walls that are somewhat thickened. Although these cells are similar to those previously described to the outside of this layer, they differ in that they are less compactly arranged, have intercellular spaces, possess thinner walls, and are oval in cross-sectional shape rather than rectangular. These five to seven layers of three types of cells evidently form the thin hard covering over the outside of the fruit. This covering assumes the function of an epicarp in the mature fruit. As was noted in the developing fruit, the epidermis of the gynophore appears to have been sloughed off and the outer layer of the young fruit consists of crushed parenchymatous mesocarp cells. It seems that the components of the epicarp of the mature fruit are merely differentiated cells whose origin is in the cells of the mesocarp of the immature stages of fruit development. The term epicarp provides a useful means of indicating the different nature of the smaller thick-walled cells of these outer layers.

Inside of the ring of five to seven cell layers of epicarp is seen an area of parenchymatous mesocarp cells. This parenchymatous region is about ten cell layers thick over the vascular bundles and about thirty to forty cell layers in depth over the "valleys" in the sclerenchymatous mesocarp. The number of cell layers in these areas is rather difficult to determine because of the crushing and obliteration of many of the cells. The cells of this region are large thin-walled parenchyma cells and are considered to be a portion of the mesocarp. The mesocarp, in

addition to the parenchymatous region, consists of a sclerenchymatous layer and the vascular bundles.

The sclerenchymatous mesocarp as seen in cross-section is structurally similar in appearance to the same area in the developing fruit. In the pericarp of the mature fruit, however, the fibrous cells are more completely developed, are larger, and have thicker walls than was the case in the developing fruit. A situation similar to that seen in the cross-sections of the developing fruit concerning the orientation of the long axes of the fibers is seen in a cross section of the pericarp of the mature fruit. Most of the fibers are oriented with the long axes parallel to the long axis of the fruit, although many are oriented with the long axes approximately perpendicular to the long axis of the fruit. The innermost one or two layers and the outermost one or two layers do not have extremely thick walls, although they are thicker than the walls of the adjacent parenchymatous cells and they are smaller in cross-sectional diameter than the parenchyma cells. The central layers of the sclerenchymatous mesocarp have very thick cell walls. All of the walls of this portion of the mesocarp retain the red color in a safranin-fast green stain and are probably lignified. While this fibrous portion of the mesocarp is somewhat more extensive under the vascular bundles, not all of the "crests" under the bundles form the fibrous flanges that project up along the sides of the vascular bundles. Some of the crests show these projections and they are similar to the ones described for the developing fruit. The maceration slides of the sclerenchymatous mesocarp often show its components to possess "saw-teeth" margins as described

by Winton (1904). As described also by Winton (1904), the cells are often of various shapes; curved or crooked in outline, with forked tips, and with arm-like projections that give them a stellate sclereid type of outline. Actually the sclerenchymatous mesocarp consists of an intergrading array of fiber and sclereid cell types. Their closely compacted association with adjacent cells oriented at almost right angles is the condition apparently responsible for the "saw-teeth" margins. The cells possess essentially simple pits that are the type usually seen in sclerenchymatous cells. The pits in these thick-walled cells are seen to have long slit-like canals, with pit chambers virtually absent. Frequently these slit-like canals are branched.

Smith (1950) stated that the peanut fruit is naturally indehiscent, but due to the incomplete formation of the sclerenchymatous mesocarp under the upper and lower surfaces, the pericarp is potentially dehiscent along two lines and is therefore a legume. The peanut fruit grows with its long axis parallel to the surface of the substratum, as shown by Smith (1950) and the term "upper surface" refers to the surface nearest the surface of the substratum, while the side farthest away from the surface of the substratum is the "lower surface." Cross-sections of the upper and lower portions of an undamaged mature pericarp were studied to determine the nature of the sclerenchymatous mesocarp at the potential lines of dehiscence. Since the commonly accepted theory of the phylogenetic derivation of flower parts depicts the carpel as a highly advanced form of spore bearing leaf that has rolled into a tube with its upper spore bearing surface to the inside, one would expect to find one line

of dehiscence in the region of the primitive "mid-rib" (dorsal side) and the other between the lateral edges (now fused) of the primitive spore bearing leaf (ventral side). By causing the fruit to dehisce by crushing it between the fingers and examining it with a 20X dissecting microscope, it was determined that the funiculi of the seeds are attached inside of the locule to the upper surface of the fruit. One funiculus was seen on each half of the dehisced pericarp, indicating that the funiculi are attached alternately along the two longitudinal vascular bundles on either side of the line of dehiscence. Consequently, the upper side of the fruit is the ventral surface, while the lower side is the dorsal surface. This is in agreement with the findings of Smith (1950). Cross-sections of the dorsal half of the pericarp verify the fact that the line of dehiscence is associated with a vascular bundle representing the midrib. Under the vascular bundle the fibrous mesocarp is weakly developed. The fibrous cells are not compactly arranged in this area as in other regions of the sclerenchymatous mesocarp. Evidently fibers are present in the area, but it appears that all that are present are arranged with their long axes parallel to the vascular bundle above the area. This fact in addition to the fact that these fibrous cells are so loosely arranged, with intercellular spaces occupying practically as much of the area as the cells, causes the weak point that is the line of dehiscence. The cross-sections of the ventral side of the pericarp show a similar region of incompletely developed sclerenchymatous mesocarp, except for the fact that this undeveloped region is not located under a vascular bundle. In somewhat the manner of the sclerenchymatous mesocarp

under a vascular bundle, the region forms a raised "crest", although no vascular bundle is to the outside of the crest area. Between the two sloping lateral areas of the crest is the loosely organized area of fibrous cells similar to those of the dorsal line of dehiscence.

The vascular bundles are essentially the same as those described for the pericarp of the developing fruit. One development that has taken place is the differentiation of fibrous caps to the outside of the primary phloem. The fibers of these caps have quite thick walls that are apparently lignified, as is indicated by the retention of safranin in a safranin-fast green stain. The macerated tissue shows these to be typical long slender fibers with pointed ends and simple pitting similar to that seen in the sclerenchymatous mesocarp. The bundles do not contain an appreciably larger number of cellular elements than were noted in the developing fruit. The elements are in a more advanced degree of differentiation than those of the developing fruit however. Even though a cambial layer is present in the bundles, its contribution to the bundles has not been extensive. Apparently the bundles still contain potentially functional phloem and xylem elements at this stage. Although tannin sacs were not seen frequently in the cross-sections of the pericarp, some were noted below the vascular bundles and above the sclerenchymatous mesocarp. These contain the now familiar homogeneous reddish-brown material. The xylem elements, as seen in the macerated tissue, consist of vessels with spiral wall thickenings and with scalariform wall thickenings. The vessels with the spiral thickenings obviously represent the first formed elements because of their small diameter and great length. A progression

in vessel types may be seen in the macerated bundles that varies from much stretched spirally thickened elements through somewhat shorter and broader elements with wall thickenings intermediate between spiral and scalariform and simple perforations placed on obliquely angular ends to short broad elements with thickenings intermediate between scalariform and scalariform-reticulate and large simple perforations that almost completely cover the virtually transverse ends.

The endocarp is to the inside of the sclerenchymatous mesocarp. In the mature fruit this region appears macroscopically as a thin layer of papery white tissue. In the cross-sections of the pericarp the layer consists of the crushed and obliterated endocarp cells that were seen in the living condition in the developing fruit. Apparently this region has been crushed by the developing seeds, and possibly the material has been utilized by the developing seeds.

The seed coat is seen in cross-section to consist of the remnants of the integumentary tissue of the ovule. The thickness of this once extensive area of cells varies from approximately 80 to 200 microns. The outermost layer of the seed coat consists of the epidermal layer of the outer integument, which has developed thickened radial walls that are thickest near the outer tangential wall and are gradually reduced in thickness toward the inner tangential wall, so that inverted cones are formed by two adjacent radial walls in the layer. The cells are approximately square, as seen in cross-section. Macerated portions of the seed coat show the surface-view of these cells and numerous simple pits may be seen in the side walls. The pits are largest to the inside of the

cells and the thickenings of the side walls show a characteristic wall that consists of finger like projections of cell wall material to the inside of the cells. Normally these epidermal cells are 3, 4, or 5 sided as seen in surface view. Winton (1904) has accurately described this layer of cells. Below the epidermal layer is seen an almost solid mass that is composed of the crushed remnants of the integumentary layers of cells. Raised areas were sometimes noted to contain about five cell layers under the epidermis that had not been completely crushed. The remnants of the vascular bundles are seen in the seed coat, embedded in the crushed integumentary cellular material. All that can be distinguished as remainders of the bundles in the cross-sections are the somewhat oval shaped bundles of xylem tracheary elements. In the macerated seed coat slides these are seen to be spirally thickened elements. Winton (1904) shows a diagram of the seed coat cross-section in which the cells are evidently not crushed and flattened to the extent of those in the material utilized in this study. He also shows a layer that he calls the inner epidermis and a one cell thick layer he labels the perisperm. When the cross-sections of the seed coat are observed carefully with the oil-immersion objective (970 X) the two layers of rectangular shaped cells may be seen on the inner surface of the seed coat. These cells are similar to the inner epidermis and perisperm described by Winton (1904). In some areas there is only one such layer of cells. It is believed by this author that in light of the observations made on the developing fruit, these two layers represent the remnants of the inner integument. Evidently the endosperm has been completely utilized

by the developing embryo. Immediately above and toward the outer epidermis from these layers is a layer of homogeneous material that has retained some of the red color of the safranin-fast green stain combination. This layer is believed to represent the cuticular layer between the inner and outer integuments.

The seed of Arachis hypogaea consists of the seed coat described above and a large embryo. The embryo occupies the entire area within the seed coat. No apparent tissue connection between the embryo and seed coat was observed in the material used in this study. The embryo consists of the short (three millimeters, more or less) hypocotyl-root axis upon which are borne the plumule, consisting of numerous leaf primordia, a small epicotyl, and two large (two centimeters in length, more or less) fleshy cotyledons. The lower end of the hypocotyl-root axis consists of the root primordium. In longitudinal sections of the dormant embryo the cells making up the hypocotyl-root axis appear to be meristematic in nature. However, the provascular tissue is obvious because of the elongate nature of the cells and the more darkly stained appearance of the protoplasts. The provascular areas in the longitudinal sections are seen to be two regions of elongate cells that are about 10 to 15 cell layers in width and that are separated by the primordial pith area. The primordial cortical area and epidermal layer are seen to the outside of each provascular area. The provascular strands may be traced into the cotyledons. In cross-sections of the hypocotyl-root axis in the region of the cotyledonary attachments, the provascular area presents the appearance of a ring of strands that is in the shape of an ellipse. The ellipse is

oriented with its long axis forming a line between the opposite cotyledonary poles. At each end of the ellipse, and near the attachments of the cotyledons are two large strands. The provascular supply to a cotyledon consists of two strands that are joined at one end to the two large strands in the hypocotyl-root axis nearest the cotyledon. The two provascular strands enter the cotyledon and there branch freely.

The cotyledons consist mainly of large (some reaching 100 microns diameter) parenchymatous cells that are polyhedral in shape and essentially isodiametric. Generally the cells are compactly arranged, although some small triangular intercellular spaces were noted. The cells possess numerous large simple pits. These cells are filled with a very large amount of stored granular food material. The cell contents have been diagrammed by Brown (1935). As is shown in his diagram, these contents are starch grains, protein granules, and oil droplets. The nature of these contents was determined in this study by using the various selective stains described herein on page 24. The adaxial surface of the cotyledons is essentially flat and the cotyledons in the seed have these surfaces pressed tightly together. A variable V-shaped groove is located on the adaxial surface. It is about in line with the hypocotyl-root axis and parallel with it, running the length of the adaxial surface. The abaxial surface of a cotyledon in cross section is seen to form a semi-circular curve. Both cotyledons pressed together in the seed form a circular cross-section. In longitudinal section the abaxial surface of a cotyledon is in the shape of a semi-ellipse. A typical epidermis covers both the adaxial and abaxial surfaces of the cotyledons. In cross-sections,

these cells are square to rectangular in shape and range in size from about 10 microns radially by 10 microns tangentially to 25 microns radially by 35 microns tangentially. In longitudinal section the cells of the epidermis are approximately 25 to 70 microns in length. These cells also contain stored food material and have thickened outer walls. As was noted by Winton (1904) this epidermis possesses stomata on the adaxial and abaxial surfaces. No experimental work was attempted in this investigation to determine whether these stomata are functional or not in the dormant seed.

The cross-sections of dormant cotyledons show an arc of provascular strands that is situated about six to eight cell layers under the curved abaxial surface and perpendicular to the plane of the sections. By observing the cross-sections of cotyledons from germinating seeds, the cells of the provascular strands may be seen in the process of differentiation. The vascular bundles of the cotyledons taken from a seed that had been germinating for nine days were still immature, although containing some apparently mature vascular elements. Most of the stored food was apparently gone from the cells of the cotyledon at this stage. The bundles consist of xylem and phloem elements, with the xylem elements to the adaxial side of the bundles. In cross-sections of a cotyledon at a point about midway between the two ends, seven vascular bundles are seen in the arc of vascular tissue.

IV

DISCUSSION

Several questions of some import have arisen as a result of this investigation that cannot be answered adequately by the examination of material prepared for utilization in the study. Pre-eminent among these questions are considerations concerning the origin of the fiber bundles of the stem and the development of the epicarp of the fruit.

It has been shown by Yarbrough (1949) and appears from the results of this study that the protophloem of the root of Arachis hypogaea develops prior to the differentiation of the phloem fibers. Further, the protophloem develops to the outside of the phloem fibers and is eventually crushed between the fibrous arcs and the pericycle. From this information, it is seen that the term "phloem fibers" is quite accurate when applied to the fibers of the root. However, in the stem the protophloem develops prior to the fibers, but to the inside of the primordial fiber bundles. This situation leads one to the conclusion that the fiber bundles of the stem are pericyclic in origin. Such a conclusion is difficult to defend when an attempt is made to identify the pericycle of the stem. Although no definite statement can be made concerning the true origin of the fibers of the stem, it appears from the developing material used in this study that the primordial fiber tissue is an integral part of the provascular strands. It is felt that further developmental studies of the developing epicotyl, coupled with detailed studies of the nature of the fiber cells, might prove fruitful in answering some of the questions about the fiber caps of the stem vascular bundles.

It seems that a more detailed developmental study of the fruit will provide more adequate information concerning the epicarp of the peanut fruit. Winton (1904) has shown an epidermis to be present on the surface of the mature fruit, whereas the information in this study tends to indicate that the epidermis of the gynophore is sloughed off during development. The fact that hair-like structures have been reported on the mature fruit by several authors, but not observed in this study, may indicate that handling of the mature fruits prior to killing and fixation has removed some of the outer layers of the fruits. The developing fruits were not subjected to the chafing and scraping action that would result from the normal handling of peanut fruits in the harvesting process though and it is felt that the crushed appearance of the outer layers of the developing epicarp is not an artifact.

Some suggestions for further study of the slippage and breakage of the seed coat that is associated with the artificial drying process are presented in order that future work may be aided by the results of this study. It seems that a developmental study of the seed coat during subterranean fructification and during the natural and artificial drying process is in order. Certainly the anatomy of the region of a natural split in the seed coat would be helpful in determining the presence of any weak areas of the seed coat. No tissue connection between the cotyledons and seed coat was noted in this study, but a developmental study of the seed coat during fructification and during drying should include certainly a parallel study of the area between the seed coat and cotyledons.

V

SUMMARY

A review of the anatomical literature concerning Arachis hypogaea is presented. Due to the fact that the anatomical work concerning the plant is limited and also to the fact that the work that has been done is often quite old and obscure, a definite need exists for research along this line.

A thorough outline of the methods used in the investigation is included in order to provide a suitable array of basic techniques for future research with the plant and its parts.

The primary root is of the normal dicotyledonous type with tetrarch xylem. Lateral roots arise in the pericycle at points that are directly in line with the four primary xylem strands. A pith is present in the primary root. The lateral roots have no pith and their xylem is diarch.

The stem is of the normal herbaceous dicotyledonous type. The arrangement of tissues is in the form of a dictyostele and a pith is present that breaks down irregularly in older stems. Secondary tissues develop an essentially continuous vascular cylinder. Older stems are actually quite "woody" due to the presence of secondary tissue.

The leaf is a normal angiospermous type exhibiting an epidermis with numerous stomata that are neither raised nor sunken on both the adaxial and abaxial surfaces. A typical palisade layer and a reduced spongy area are present in the leaf, in addition to a layer of large thin walled water storage cells.

The flower is perigynous with a papilionaceous arrangement of the petals, the hypanthium contains the vascular supply of the petals, sepals, and stamens. The ovary usually contains two ovules in the locule. It is the elongating base of the ovary that gives rise to the gynophore. The gynophore anatomy is similar to that of the stem, except that no lateral branches are present.

The fruit develops underground and the mature pericarp consists of a parenchymatous section of the mesocarp with an outer layer that serves the function of an epicarp, a hard fibrous mesocarp that gives strength and rigidity to the structure, and an endocarp that is composed of crushed parenchymatous cells. Usually two large seeds are located within the locule of the pericarp. The seed coat is apparently composed of the crushed remains of the integuments of the ovule. The embryo possesses two large fleshy cotyledons whose cells contain a large quantity of stored food in the form of starch grains, protein granules, and oil droplets.

VI

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Although often not specifically cited, the works of Esau (1953) and Eames and McDaniels (1947) were drawn upon throughout the study.

To others too numerous to mention, who have aided materially in the completion of this work by their suggestions and criticisms, thanks are due.

VII

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VIII

VITA

James Robert Brennan was born in Cranfordsville, Indiana on November 14, 1930; the first son of James E. and Martha Truax Brennan. In 1939 he moved with his family to Portsmouth, Virginia. He was graduated from St. Joseph's Academy in Portsmouth, Virginia in June 1947. Having completed the requirements for the Bachelor of Science Degree in December 1951 at Virginia Polytechnic Institute and one quarter of graduate work in Botany at the same institution, he was called to active duty with the U. S. Army Ordnance Corps as a Second Lieutenant. Upon completion of twenty and one-half months active duty with the Army in the United States, Japan, and Korea, he was returned to inactive reserve status. He resumed graduate studies in Botany at Virginia Polytechnic Institute in January 1954. From January 1954 until June 1954, he held the position of Instructor in Biology at Radford College, Radford, Virginia. He will receive the Master of Science Degree in Botany in June 1955. He was married to Jean Gardner in December 1953 and has one daughter, Catherine Ann. As a member of Alpha Psi Chapter of Phi Sigma he has served in the capacities of delegate to the National Biennial Convention in 1954 and Chapter Secretary in 1954 and 1955.

James R. Brennan

Table 1

Dehydration, Infiltration and Embedding Schedule

Solution	Time in Solution	
	A	B
Tertiary Butyl Alcohol 10% 95% Ethyl Alcohol 40% Distilled Water 50%	48 hours	24 hours
Tertiary Butyl Alcohol 20% 95% Ethyl Alcohol 50% Distilled Water 30%	48 hours	24 hours
Tertiary Butyl Alcohol 35% 95% Ethyl Alcohol 50% Distilled Water 15%	48 hours	24 hours
Tertiary Butyl Alcohol 75% Absolute Ethyl Alcohol 25%	48 hours	24 hours
Tertiary Butyl Alcohol 100%	24 hours	24 hours
Tertiary Butyl Alcohol 100%	24 hours	24 hours
Tertiary Butyl Alcohol 100%	24 hours	24 hours
Tertiary Butyl Alcohol 50% Paraffin Oil 50%	48 hours	24 hours
Tertiary Butyl Alcohol Paraffin Oil * Tissuemat	24 hours	24 hours
Tissuemat	24 hours	24 hours
Tissuemat	24 hours	24 hours
Tissuemat (This used for embedding)	24 hours	24 hours

* Equal portions of each of the substances listed were placed in the oven at 50° to 60°C.

Table 2

Conant's Quadruple Stain

Staining Schedule for Paraffin Sections

Solution	Time in Solution
100% Xylene	1 to 1½ minutes
100% Xylene	30 seconds to 1½ minutes
75% Xylene 25% Absolute Ethanol	"
50% Xylene 50% Absolute Ethanol	"
25% Xylene 75% Absolute Ethanol	"
Absolute Ethanol	"
Absolute Ethanol	"
95% Ethanol	"
90% Ethanol	"
85% Ethanol	"
80% Ethanol	"
75% Ethanol	"
70% Ethanol	"
60% Ethanol	"
50% Ethanol	"
0.5% Safranin in 50% Ethanol*	24 hours
Distilled Water	1 minute
Saturated Aqueous Solution of Crystal Violet	"
Distilled Water	"
50% Ethanol	30 seconds to 1½ minutes
60% Ethanol	"
70% Ethanol	"
80% Ethanol	"
90% Ethanol	"
95% Ethanol	"
Absolute Ethanol	"
Absolute Ethanol	"
0.1% Fast Green in Absolute Ethanol	10 dips (app. 15 seconds)
Saturated Orange G in Clove Oil	2 to 3 minutes
Saturated Orange G in Clove Oil	"
Saturated Orange G in Clove Oil	"
100% Xylene	45 seconds to 1 minute
Canada Balsam	Permanent Mount

*1.0% Safranin in methyl cellosolve (Johansen 1940) was substituted at one point in the study and the material was retained in this solution for 48 hours.

Table 3

Safranin and Fast Green Stain

Staining Schedule for Pericarp and Seed Coat Sections

Solution	Time in Solution
100% Xylene (in petri dish)	1½ minutes
1% or 2% Celloidin in 50% Ethyl Ether-50% Absolute Ethanol*	Until Solidification (15-20 min.)
50% Ethanol	1 minute
0.5% Safranin in 50% Ethanol	18-24 hours
50% Ethanol	30 seconds
60% Ethanol	"
70% Ethanol	"
75% Ethanol	"
80% Ethanol	"
85% Ethanol	"
90% Ethanol	"
95% Ethanol	"
0.1% Fast Green in 95% Ethanol	5 minutes
95% Ethanol	"
Absolute Ethanol	30 seconds
Absolute Ethanol	"
50% Ethyl Ether-50% Absolute Ethanol (in petri dish)	Until Celloidin Removal (5-20 min.)
100% Xylene (in petri dish)	1 minute
Canada Balsam	Permanent Mount

*Applied to surface of slides with a dropper

Table 4

Safranin Stain

Staining Schedule for Macerated Tissues

Solution	Time in Solution
Distilled Water	30 seconds
10% Ethanol	"
20% Ethanol	"
30% Ethanol	"
40% Ethanol	"
50% Ethanol	"
0.5% Safranin in 50% Alcohol	8-10 hours
50% Ethanol	30 seconds
60% Ethanol	"
70% Ethanol	"
75% Ethanol	"
80% Ethanol	"
85% Ethanol	"
90% Ethanol	"
95% Ethanol	"
Absolute Ethanol	"
Absolute Ethanol	"
25% Xylene-75% Absolute Ethanol	"
50% Xylene-50% Absolute Ethanol	"
75% Xylene-25% Absolute Ethanol	"
100% Xylene	"
100% Xylene	1 minute
Canada Balsam	Permanent Mount

EXPLANATION OF PLATES

Plate 1 - Diagrammatic cross-section of the root of Arachis hypogaea showing four lateral roots and basic tissue areas.

Plate 2 - Diagrammatic three dimensional view of the stem of Arachis hypogaea showing the basic tissue areas.

Plate 3 - Diagrammatic cross-section of the flower of Arachis hypogaea, at the level of an ovule, showing the basic tissue areas.

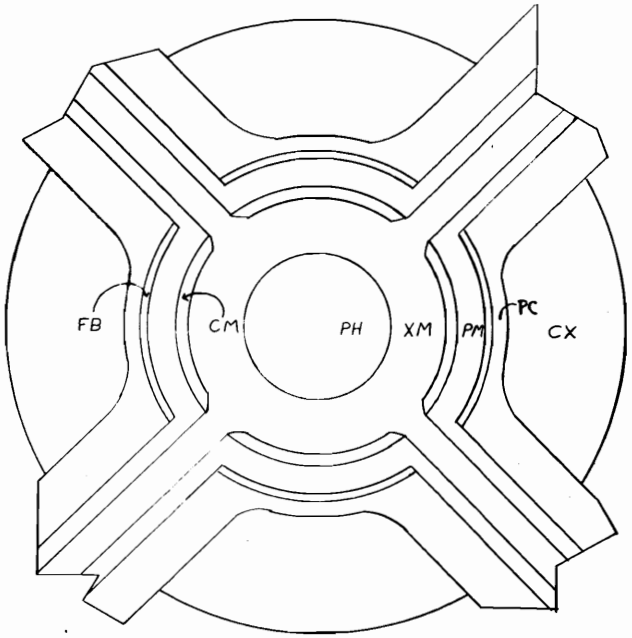
Plate 4 - Diagrammatic cross-section of an ovule of Arachis hypogaea, as it appears in a gynophore that has penetrated the soil surface, showing the basic tissue areas.

Plate 5 - Diagrammatic cross-section of a mature fruit of Arachis hypogaea showing the basic tissue areas.

Plate 6 - Figures representing some cell types seen in Arachis hypogaea: (A) Section of the epidermis of a cotyledon showing a stoma located above an intercellular space (IN). 300 X. (B) Section of stem epidermis showing a stoma and several layers of collenchymatous cortex. 300 X. (C) Cells from a cotyledon showing characteristic pitting in the two cells to the right and copious food storage in the cell to the left. 300 X. (D) Section of the upper epidermis of a leaflet showing a stoma and some palisade cells. 300 X. (E) Cross-sectional view of a portion of fibrous sheath around vascular tissues in the petiolule. 300 X.

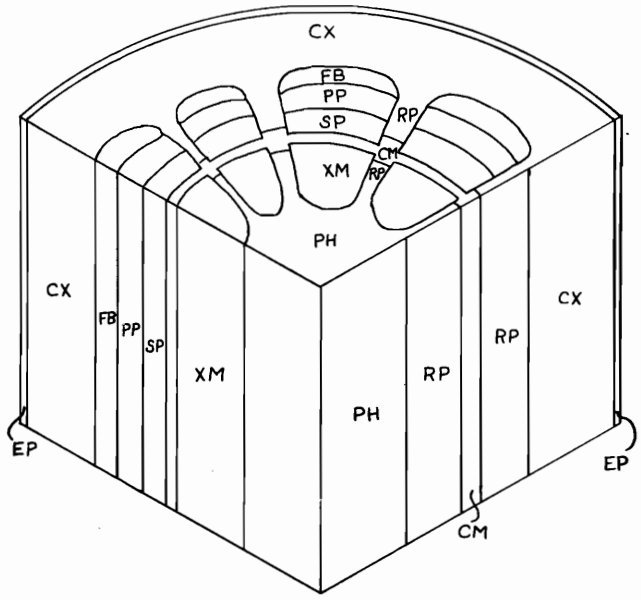
Plate 7 - Figures representing some cell types seen in Arachis hypogaea: (A) A portion of the epidermis of an elongating gynophore as seen in longitudinal section. 300 X. (B) A portion of the epidermis of the seed coat as seen in cross-section. 300 X. (C) A surface view of some epidermal cells of the seed coat. 720 X. (D, E, and F) Cells from the sclerenchymatous mesocarp of the pericarp. 300 X.

PLATE 1



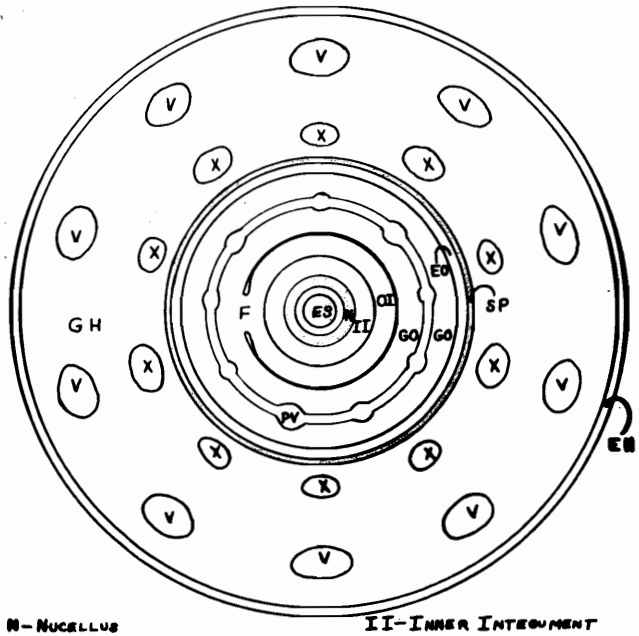
CM- CAMBIUM AND IMMATURE CELLS
CX- CORTEX
EP- EPIDERMIS
FB- ARC OF PHLOEM FIBERS
PC- PERICYCLE AND CORK
PH- PITH
PM- PHLOEM
XM- XYLEM

PLATE 2



CM- CAMBIUM AND IMMATURE CELLS
CX- CORTEX
EP- EPIDERMIS
FB- FIBER BUNDLE
PH- PITH
PP- PRIMARY PHLOEM
RP- RAY PARENCHYMA
SP- SECONDARY PHLOEM
XM- XYLEM

PLATE 3



N- NUCELLUS

EH- EPIDERMIS OF HYPANTHIUM

EO- EPIDERMIS OF OVARY

ES- EGG SAC

F- FUNICULUS

GO- OVARY GROUND TISSUE

II- INNER INTEGUMENT

OI- OUTER INTEGUMENT

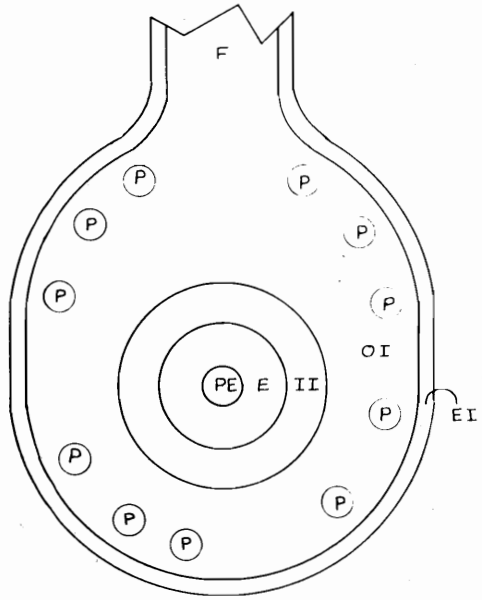
PV- OVARY PROVASCULAR AREA

SP- AIR SPACE

V- PERIANTH VASCULAR TRACE

X- STAMEN VASCULAR TRACE

PLATE 4



E- ENDOSPERM

EI- EPIDERMIS OF OUTER INTEG.

F- FUNICULUS

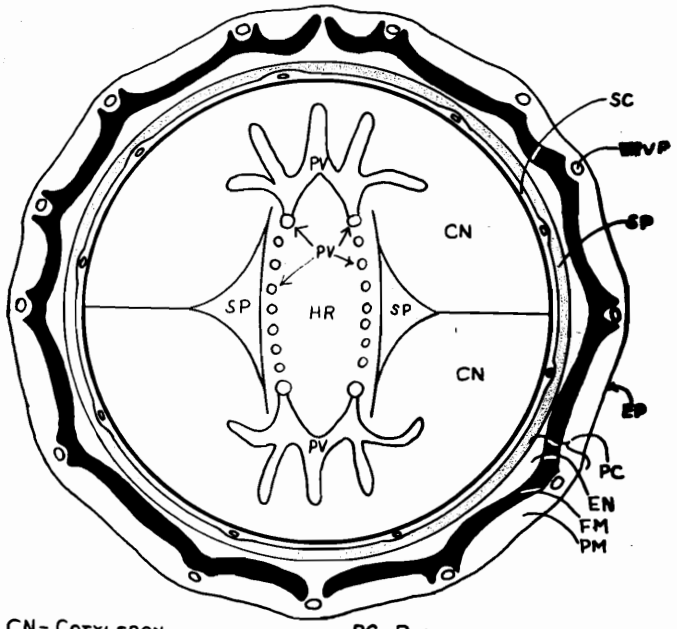
II- INNER INTEGUMENT

OI- OUTER INTEGUMENT

P- PROVASCULAR STRAND

PE - PROEMBRYO

PLATE 5



CN- COTYLEDON

EN- ENDOCARP

EP- EPICARP

FM- FIBROUS MESOCARP

HR- HYPOCOTYL-ROOT AXIS

PC- PERICARP

PM- PARENCHYMATOUS MESOCARP

PV- PROVASCULAR STRAND

SC- SEED COAT

SP- AIR SPACE

VP- PERICARP VASCULAR BUNDLE

PLATE 6

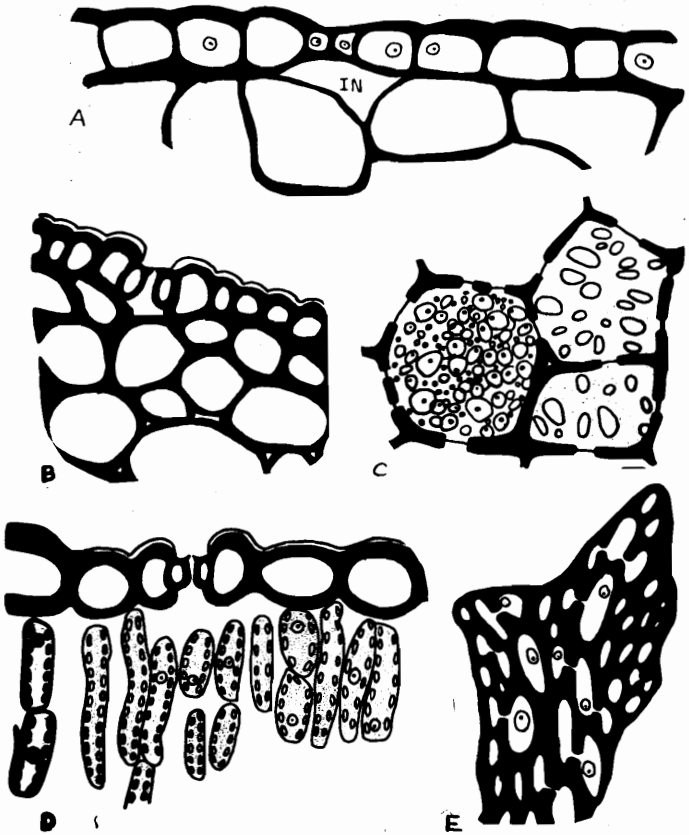
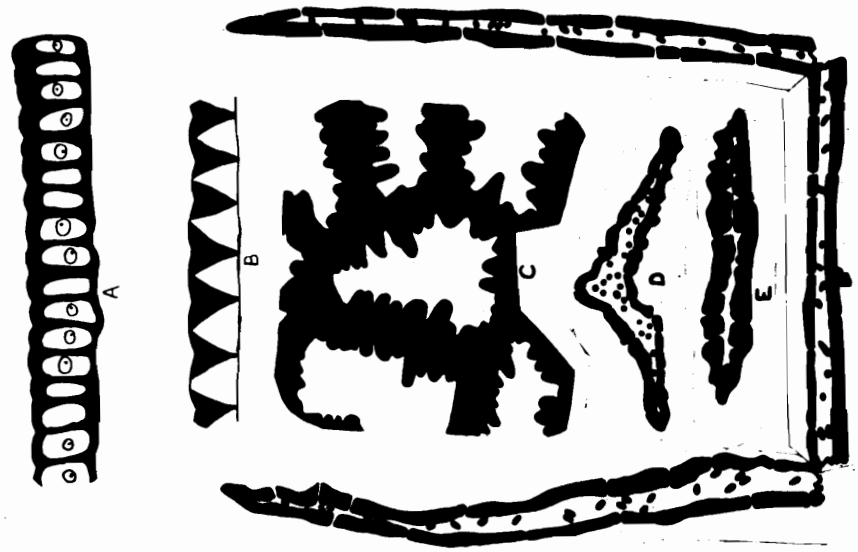


PLATE 7



EXPLANATION OF PHOTOMICROGRAPHS

Photomicrograph 1 - Portion of cross-section of primary root at level of highest lateral roots. Refer to Plate 1 for explanation of symbols. (EN - Endodermis). 120 X.

Photomicrograph 2 - Portion of cross-section of primary root at level of highest lateral roots showing juncture of lateral root. Refer to Plate 1 for explanation of symbols. (LR - Lateral Root, PE - Pericycle, EN - Endodermis). 120 X.

Photomicrograph 3 - Portion of cross-section of stem. Refer to Plate 2 for explanation of symbols. (CC - Collenchymatous Cortex, PC - Parenchymatous Cortex, TX - Tannin Filled Xylem Element). 120 X.

Photomicrograph 4 - Portion of longitudinal section of stem. Refer to Plate 2 for explanation of symbols. (TP - Tannin Sac in outer region of pith). 120 X.

Photomicrograph 5 - Portion of cross-section of leaflet in region of mid-rib. (TP - Tannin Sac, SE - Bundle Sheath Extension, UE - Upper Epidermis, PP - Palisade Parenchyma, WS - Water Storage Cells, LE - Lower Epidermis, FB - Fibrous Bundle, PM - Phloem, XM - Xylem). 120 X.

Photomicrograph 6 - Portion of cross-section of leaflet near edge. (ST - Stoma, SE - Bundle Sheath Extension, UE - Upper Epidermis, PP - Palisade Parenchyma, WS - Water Storage Cells, LE - Lower Epidermis, VB - Vascular Bundle, SP - Spongy Parenchyma). 120 X.

Photomicrograph 7 - Cross-section of flower at level of ovule. See Plate 3 for explanation of symbols. (TP - Tannin Sac). 120 X.

Photomicrograph 8 - Longitudinal section of elongating gynophore through region of ovules. See Plate 4 for explanation of symbols. (AB - Line of Abscission of Style, EG - Epidermis of Gynophore). 120 X.

Photomicrograph 9 - Portion of cross-section of expanding gynophore tip in region of ovule. See Plate 4 for explanation of symbols. (No provascular strands seen in this photomicrograph). (GE - Endocarp of Gynophore). 120 X.

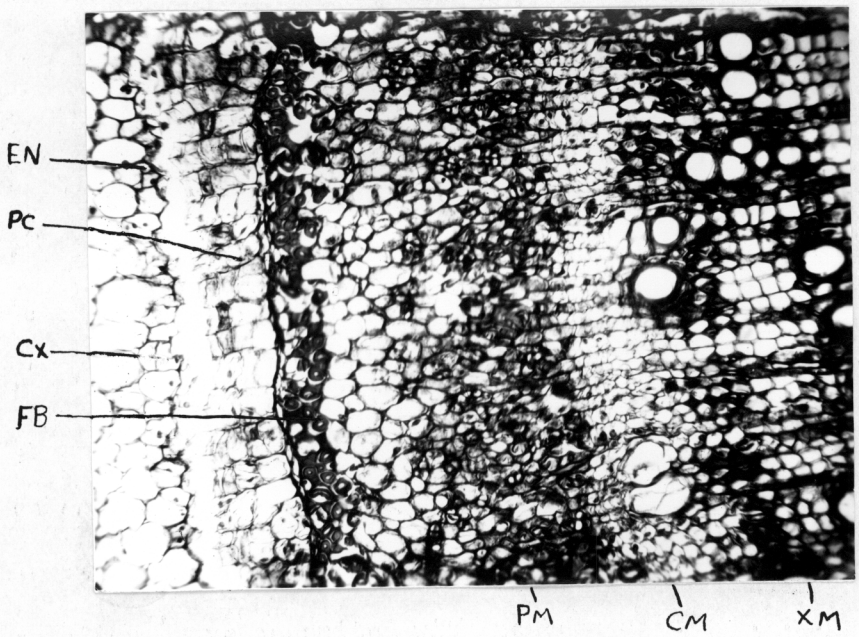
Photomicrograph 10 - Portion of pericarp of mature fruit in region of a vascular bundle. See Plate 5 for explanation of symbols. 120 X.

Photomicrograph 11 - Portion of pericarp of mature fruit in region of dorsal "mid-rib". See Plate 5 for explanation of symbols. 120 X.

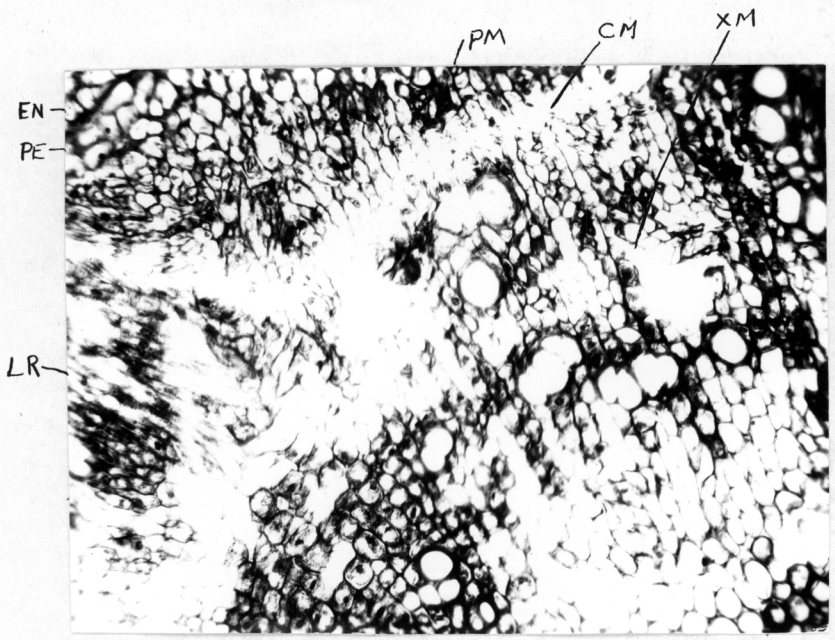
Photomicrograph 12 - Portion of cross-section of seed coat in region of vascular bundle. (EC - Epidermis of Seed Coat, EV - Vascular Bundle, ER - Uncrushed Region of Parenchymatous Cells). 120 X.

Photomicrograph 13 - Portion of cross-section of cotyledon in region of adaxial surface (CE - Epidermis, CS - Stoma, CF - Food Storage Cells). 120 X.

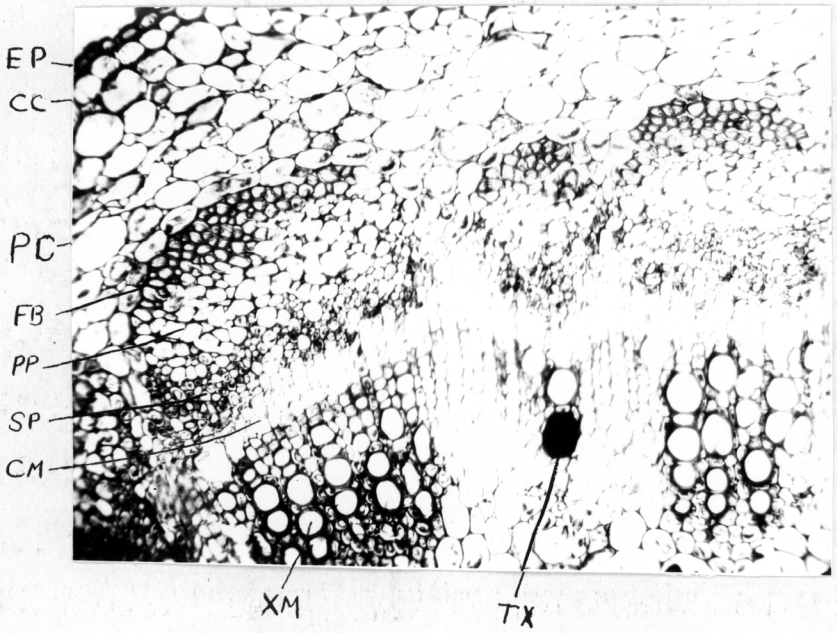
Photomicrograph 1



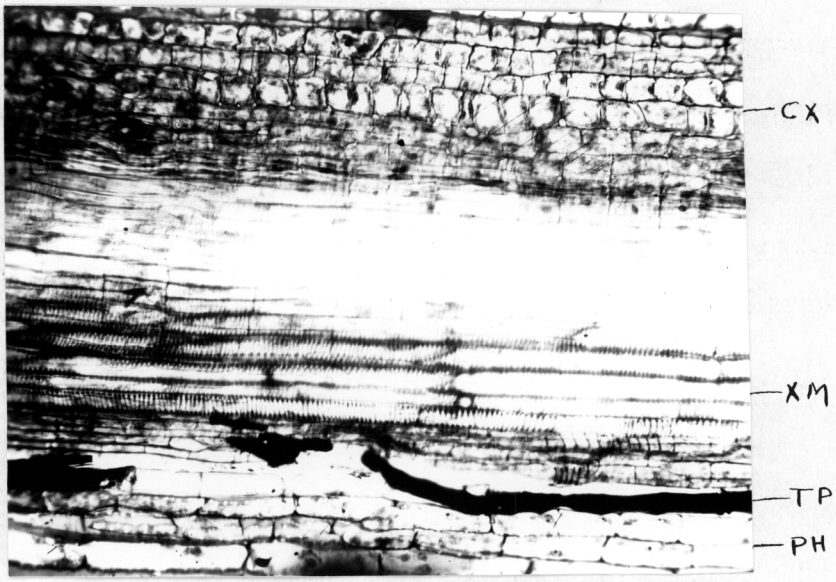
Photomicrograph 2



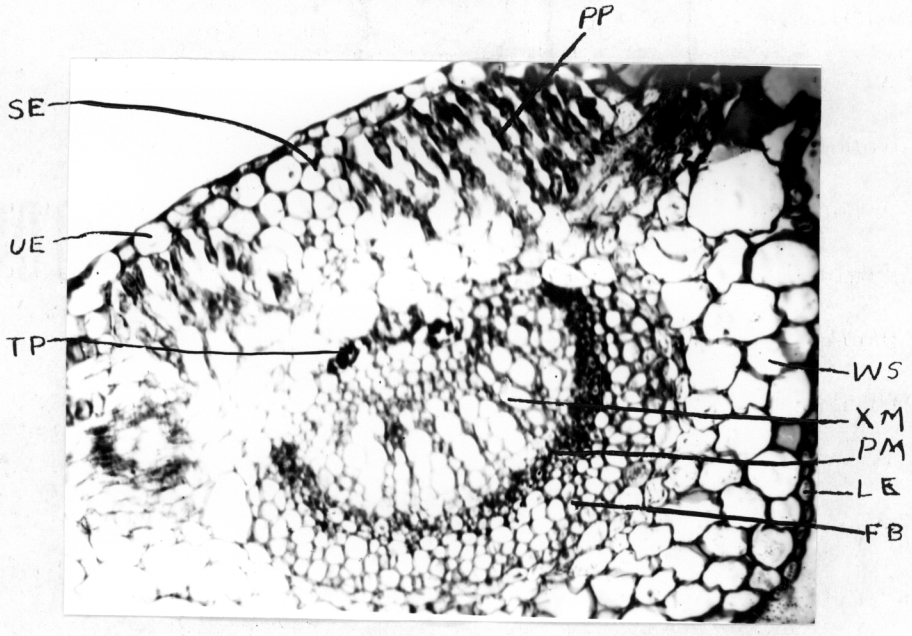
Photomicrograph 3



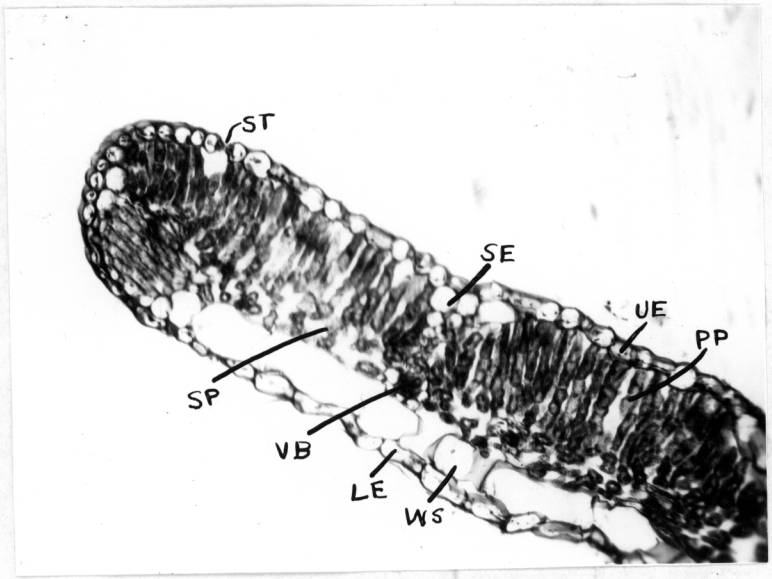
Photomicrograph 4



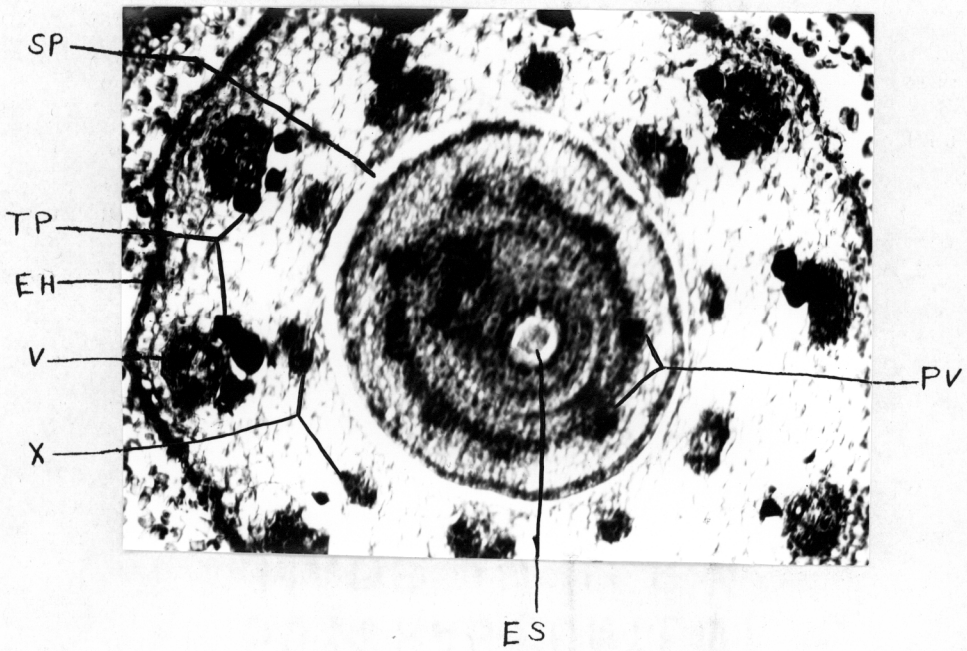
Photomicrograph 5



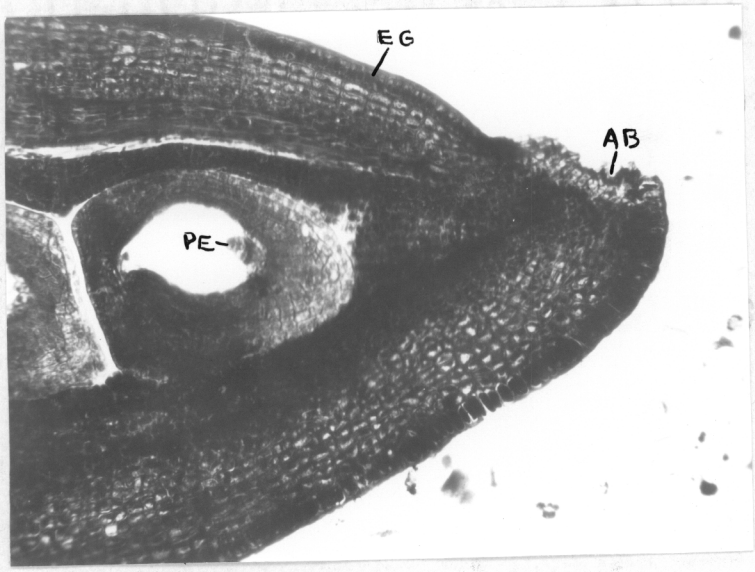
Photomicrograph 6



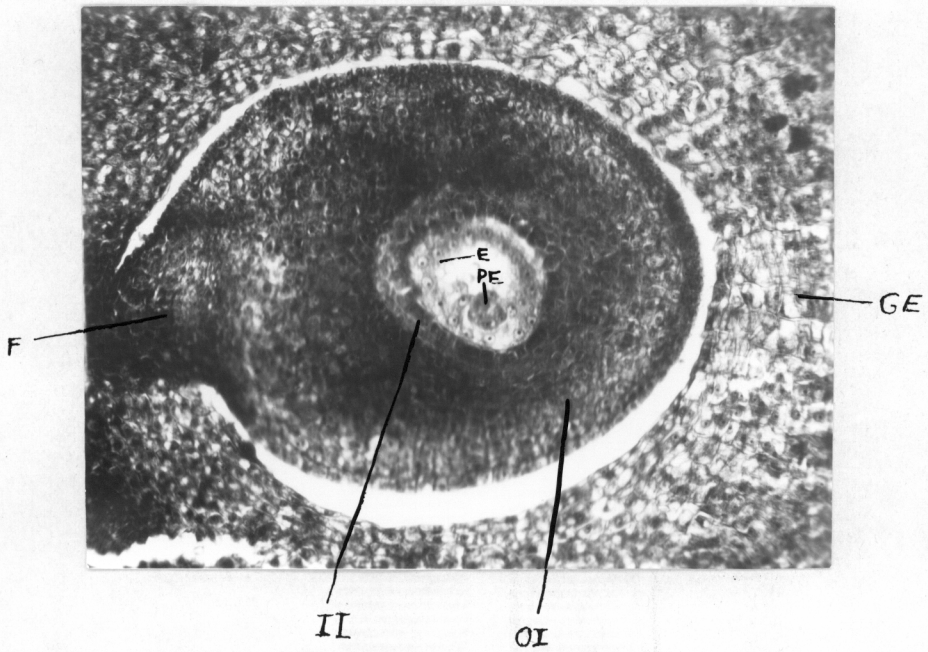
Photomicrograph 7



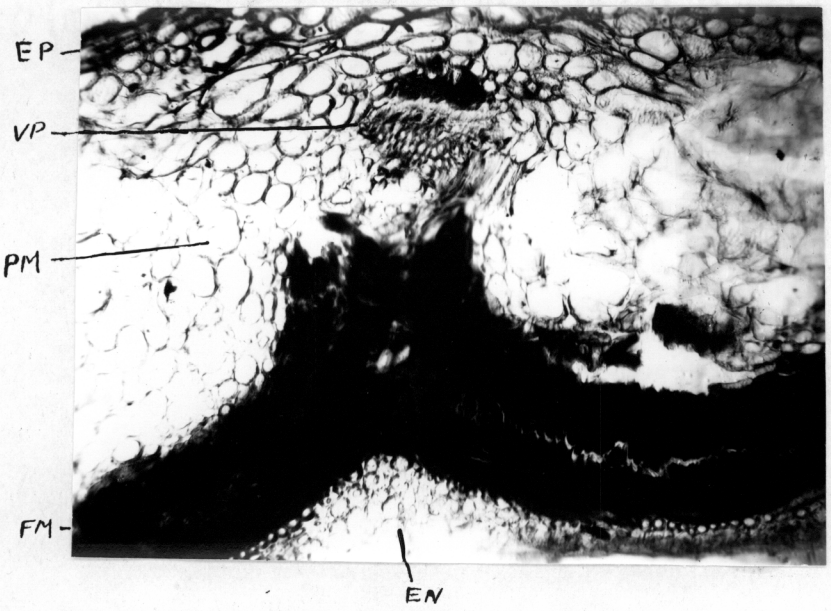
Photomicrograph 8



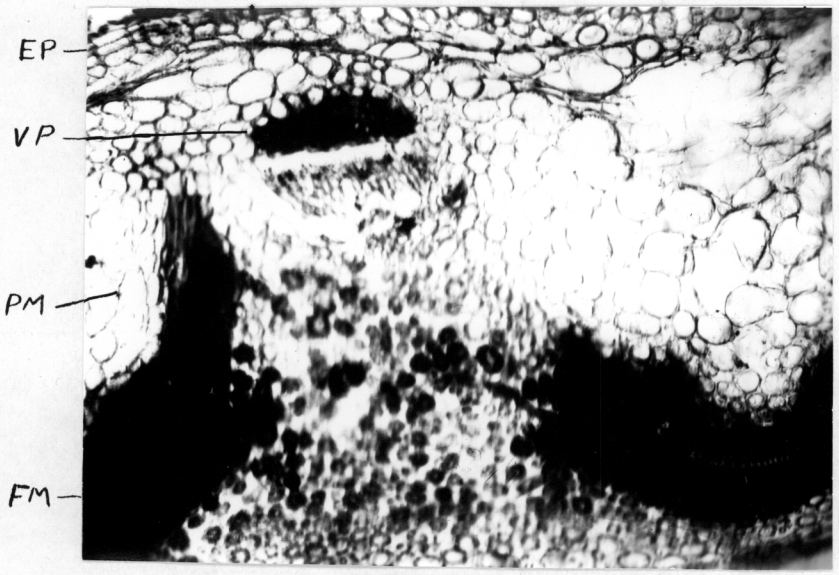
Photomicrograph 9



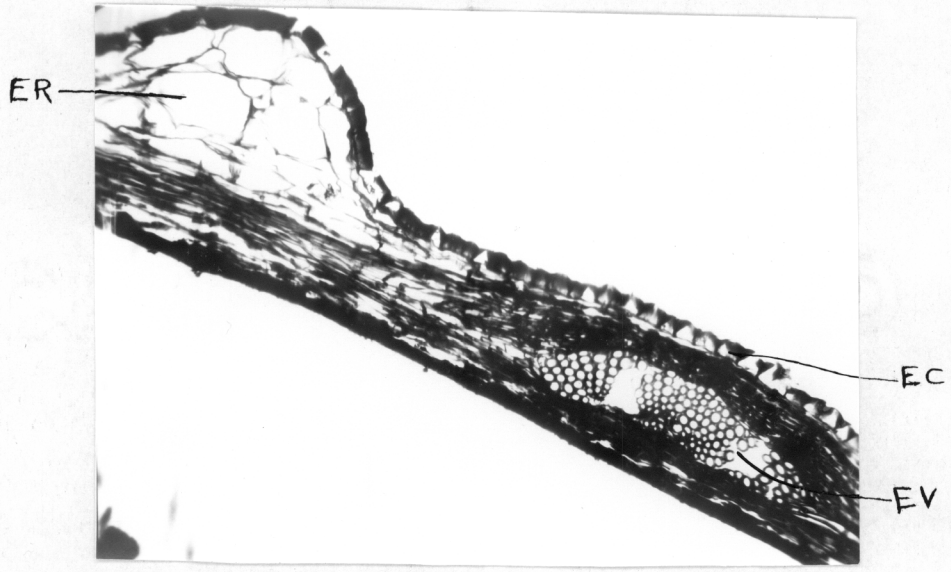
Photomicrograph 10



Photomicrograph 11



Photomicrograph 12



Photomicrograph 13