A Morphological and Cytological Study

of

Styles and Stigmas

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Science

by

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Introduction.

The observations on styles and stigmas in the following paper are based upon material grown both in the greenhouse and in the field. Investigations were begun in connection with the work of M.J.Dorsey on Sterility in the Plum. The purpose of the work was to study the types and characters of stigmas and styles to determine whether any relation existed between these structures and sterility.

The present paper contains the descriptions of styles and stigmas in or near the receptive stage. It constitutes the first phase of a more detailed study in which it is proposed to trace the development of these structures up to the formation of the abscission layer, and to determine the chemical composition of various substances present.

Methods.

The material was collected at or near the time the stigma was in the receptive condition. The fixing solution used was medium chrom-acetic fixative. The pistil was removed from the flower, and if of small size the entire pistil was taken; but if large only the style and stigma were used.

Sections were cut 10 microns in thickness. They were stained with Heidenhain's iron-alum-haematoxylin, using Yamanouchi's Schedule with only slight variations

Detailed drawings were made of groups of cells from representative portions of these structures and of representative types, while photomicrographs were made of the styles, stigmas, and special gland-like cells.

Longitudinal sections were used for the detailed studies. These studies were supplemented with a study of cross sections to determine the position and manner of distribution of various tissues. The descriptions refer to longitudinal sections unless otherwise stated.

Historical.

There has been much discussion and diversity of opinion concerning the nature, history, and development of the carpel. Coulter and Chamberlain (3) state that its history is unknown and that although it is easy to imagine that it has been derived from open carpels like those found among the gymnosperms, no clear intermediate stages have been found. They also state that, "The style is definitely related, in its varying form and length to the problem of pollination, and upon it the stigmatic surface is developed in various ways. This surface is increased in area by the enlargement of the apex of the style, by its branching, or by being developed.

"One of the essential features of the structure of the carpel is the provision for the progress of the pollen-tube from the receptive surface to the sporangium or even to its micropyle. A specialized and continuous nutritive tissue connects these two extremes, often confused in the sporangial chamber with the placenta, in the style called conductive tissue and upon its surface the stigma, but forming one continuous tissue system, well named conducting tissue.

"In case of hollow styles, as in Lilium, Butomus, Agave, Erythronium, Viola, Campanula, Sardoes etc., the conductive tissue lines a canal as a glandular layer, or in some cases, as in Anagallis, fills up a hollow style; but in most cases the style is solid, with the conductive tissue as an axial strand. In case a single style is connected with two or more sporangial chambers, the strands of conductive tissue branches into each chamber."

However according to Le Macout and Decaisne (5), the style is formed from the upper part of the carpellary leaf, narrowed and rolled up in such a manner as to form a sort of longitudinal canal. This canal, they say, is occupied by a somewhat compact parenchyma called conductive tissue, which spreads out at the top or one the sides of the style forming a spongy surface constituting the stigma. They state that this same tissue descends from the style into the cavity of the ovary, and runs along the placentae to the micropyle of the ovules.

In 1857 Payer (6) said, "Botanists are far from being agreed on what one may call stigma. For myself, I consider as stigma only that part of the style or of the style branches which is covered with stigmatic papillae."

Capus, M.G. (2) applies the term stigma to that part of the style which is formed exclusively by the overflowing at the surface of the conductive tissue.

Behrens (1) considered as stigma only that part of the style which is differentiated into papillae. This is the use of the term, stigma, accepted by most botanists at the present time.

Behrens (1) in 1875 published the first noteworthy study of styles and stigmas. In this work he discussed the general structure of these organs as a whole, described somewhat in detail the histological characters of a number of species ranging over several families, and established certain types both of styles and stigmas. Very little attention, however, was given to the cytological side of the problem.

Capus (2) 1878 published the next important study which has been made of styles and stigmas. His work deals entirely with the conductive tissue. He discusses the formation of this tissue and deals with its structure in the ovary, the style, and on the stigma. He also devotes some attention to physiological considerations on the function of the conductive tissue. He applies the term conductive tissue to that tissue which is composed of elements usually elongated, loosely united and delicate, of which the assemblage forms a cylinder which occupies the center of the style or covers the wall of a central stylar canal.

From his observations, he concluded that

1. The conductive tissue may be constituted by the modification of certain cells from either the epidermis alone or the epidermis and the fundamental tissue, or be a newly formed tissue resulting from --

   a. the tangential division of the epidermis

   b. the cells of the periblem

2. The origin of the conductive tissue is double for each carpel.
3. The conductive tissue may line a stylar canal or itself constitute a solid conductive cylinder.
4. The solid conductive tissue is formed by the welding of the opposite edges of the original canal.
5. The solid conductive tissue becomes ready to conduct the pollen tubes by the gelatinization of the middle walls of its elements.
6. The stylar canal may be simple or divided.
7. The true stigma is formed exclusively by the conduction tissue either by modification or from new formation.

8. The volume of conductive tissue is in proportion to the number of ovules to be fertilized, because it is in proportion to the number of pollen tubes which descend into the ovary to fertilize these ovules.

9. The extent of the stigmatic surface is determined by the volume of the conductive tissue.

10. The stigma determines then the number of grains of pollen which may germinate and the number of ovules which may be fertilized.

Capus, like Behrens, discusses mainly histological structures and limits cytological observations to the comparison of density and appearance of cells contents in neighboring tissues.

Discussion.

Style.

The style is that portion of the carpel which surmounts the ovary and bears at its upper end the stigma which is a modification of the conductive tissue of the style.

In the majority of cases the style is cylindrical, but it may be angular, flattened, etc. Sometimes the union of the carpels may be traced on the style.

The style may be very long, short or absent in which case the stigma is said to be sessile on the ovary. The stigma, as has been previously stated, is only that portion of the conductive tissue which is modified in the form of papillae. Thus as we shall see, the style terminates in many forms and the stigmatic area occupies various positions on the style.

The gross structure and form of the style have been studied extensively, but aside from the two papers briefly reviewed no work has been published giving the details of its nature.

The style is composed of epidermis, ground tissue, vascular tissue and conductive tissue. These tissues will be discussed in the above named order.

Epidermis.

The epidermal cells in general are rather uniform in size and shape. They vary from almost square to much elongated rectangular cells. Behrens (1) said that in longitudinal section they are everywhere rectangular, elongated or six sided, but in cross section are round, elliptical, quadrangular etc.

The outer walls are covered with cuticle which varies from a very thin, uniform layer to very thick irregularly disposed layers. Behrens (1), observed that directly under the stigma in Polygala the cuticle is laid down in two layers; that in Thydaea it is tagentially striated and in Helianthemum it is radically striated.

The edge of the cuticle may be either wavy or even but it is usually rather uniform.

In the species studied the greatest variation in disposition of cuticle was found in Agapanthus sp. in which the outer walls of the epidermis were lined with a thick layer of cuticle which is wavy or scalloped at the inner edge. Heavily cutinized epidermal cells were also found in Ismene Calathena, Plate 18, Fig. 1, in Mamillaria pusilla Plate 25, Fig. 2, and in Arisaema triphyllum Plate 13, Figs. 1 and 2.

The epidermal cells may or may not be modified to form hairs or other outgrowths. These structures are mostly unicellular but may be multicellular. They may be glandular and contain oil or other substanced.

The most complex modifications of epidermal cells were found in Juglans cinerea, Malvaviscus Mollis, Hibiscus sp. and in Malus ioensis. Juglans cinerea has two kinds of hairs. One type is composed of a multicellular base terminated by a single, elongated, cone-shaped, glandular hair. The other type is composed of a stalk-like cell terminated by a multicellular tip. In Malvaviscus Mollis the epidermal cells divide to form elongated hairs which in the material studied varied from a few to twenty-three cells. The epidermal hairs of Hibiscus sp. are few to several-celled, and are densely filled with oil globules as are the unmodified epidermal cells. Malus ioensis has such numerous elongated hairs that they appear as a dense covering over the style.

In some cases as in Ribes sp. the epidermal cells are made conspicuous by the greater density of their cytoplasm as compared with that of the neighboring parenchymatous tissue, but more often their differentiation is due to the cells being more regular and thicker walled than the parenchymatous cells.

The cytoplasm of the epidermal cells is usually rather scant, vacuolate, and more abundant about the walls. The nuclei are not unusual in any respect except in Arisaema triphyllum, Plate 13, Figs. 1, 2 whose epidermal cells have very conspicuous, large, spherical nuclei thus causing the epidermal cells to stand out very conspicuously.

The epidermal cells often contain oil glands, and other substances found in the other tissues of the plant.

In most cases the epidermis is found only as the outermost layer of the style and in all cases studied it was but one layer in thickness. However in Ribes sp. Plate 21,Figs.1,2,the epidermal layer appears to line a stylar canal, but in fact is only surrounding the partially fused styles; because in Ribes there is a one-celled ovary, with two parietal placentae, and two distinct or united styles. In this species the fusion of style is so incomplete that the epidermal cells remain and each style has its own conductive tissue as is clearly shown in the figure.

Ground Tissue

The ground tissue, in which the fibro-vascular bundles are variously though usually concentrically arranged, lies directly within the epidermis. It may comprise a large proportion of the style, as in the Araceae, a very small proportion as in Ribes sp. Plate 21, Figs.1,2,but most often it occupies from one-half to one-third of the diameter of the style.

The cells of this tissue are parenchymatous in nature. They vary from slightly to extremely irregular, and contain intercellular spaces especially opposite the end walls.

Very often some of the cells break down leaving spaces several times large than the cells. These spaces may or may not contain raphides, and they are very abundant in the Araceae. In Aquilègia canadénsis Plate 25,Fig. 1, are found large gland-like structures which are in some cases empty but more often densely filled with oil globules.

The cytoplasm is less dense than that of the neighboring conductive tissue, but usually contains more starch grains, oil globules, resin and tannin when these are present in any of the tissue.

Vascular Tissue

As already stated the vascular bundles lie in the ground tissue and are variously arranged although usually concentrically. According to Behrens (1) their number and position correspond in general to the scheme of the flower. Therefore, since in most cases the style corresponds to the middle portion of the carpel, there are as many bundles as there are carpels in the ovary. In other cases, as in the Monocotyledons there are three, rarely two, small bundles at the side of each main bundle. In some cases there are two bundles to each style branch.

In the cases observed, it was found that the vascular tissue lies, in most cases, next to the conductive tissue. However the bundles bend outward near the bases of the style and pass into the ovary near the center of its wall.

Conductive Tissue

As already stated Coulter and Chamberlain (3) emphasize the fact that this tissue is one continuous system and lament that it has been given separate names according to its position thus "diverting attention from the continuity of this tissue." Behrens (1) states that the conductive tissue seemed to a common tissue in styles, and with but few exceptions it passes abruptly into the ground tissue. He finds that Tilia Europaea and Phyteuma spicatum are exceptions for in these species there is a gradual transition in passing from the ground tissue to the conductive tissue.

He further states that in Musa ferruginea the conductive tissue is absent, but that even in the style of this species there are eight to nine rows of cells which run parallel to the long axis of the style and in cross section are from four to six-sided. These cells are filled with a brown liquid in which there are a few solid granules. This substance gives the reaction for mucilage and tannin. He thinks that these cells are at least physiologically if not morphologically equivalent to conductive tissue. In the forms investigated in this study none showed the absence of conductive tissue.

Behrens (1) finds the cells of the conductive tissue to be somewhat long rectangles in longitudinal section, but circular with small three-corned spaces between them in cross section. Also, that the walls of this tissue are thicker than those of the ground tissue. That the cells are arranged in longitudinal rows which may not touch each other at all, but are embedded in a slimy layer which is found not only in the canal but between the cells which line the canal. The longitudinal walls are very loosely joined, and separate from each other easily while the cross walls do not. He also states that the conductive tissue is uniform throughout where it does occur. Juglans cinerea, as we shall see, is an exception to this condition. He sums up the physiological functions of the conductive tissue as follows: (1) In plants which do not have a stylar canal the pollen tube must force its way through the stylar tissue, and since the conductive tissue is loosely arranged, it becomes the place for this. The ease with which the longitudinal walls separate facilitates the passage of the pollen tubes through this tissue. (2) If a stylar canal is present then the outer layers of the conductive function to secrete a slimy, sticky substance which through adhesion conducts the pollen tubes to the ovary. If papillae project into the stylar canal, as is the case in many plants, they have the same function as the outer layer of the conductive tissue.

Capus (2) like Behrens concludes that the conductive tissue is composed of loosely arranged cells whose walls are considerably thickened. This thickening he says may be such that the conductive tissue acquires the appearance of true collenchyma as in the Ranunculaceae. He concludes that in general the conductive tissue acquires all the properties which render it apt in conducting the pollen tubes at the time of the impregnation of the stigma.

As remarkable types of conductive tissue he describes the following species. Deherainia smaragdina has at the base of its style conductive tissue composed of from five to eight layers of cells which have very thick walls and which are densely filled with chlorophyll. These cells are covered by a row of spidermal cells in which the radial walls, little thickened in their lower half, thicken abruptly and become collenchyma-like. These cells secrete abundant mucilage which is dense and granular, and which nearly fills the entire stylar canal in which one may find a large number of pollen grains. A mucilaginous secretion was found in the stylar canal of Fagelia and of Symphytum echinatum also.

In Gesneria elongata the middle lamella of the cells of the conductive tissue becomes gelatinified in such a nammer that the cells seem to be embedded in an intercellulary substance. The cells are filled with grains of starch.

He finds that the stylar canal may be lined with compound papillae as in Reseda alba, but that the presence of simple papillae as in Asclepias, Forsythia syspensa, and Polemonium is much more frequent.

Of the species studied in this investigation all had an open canal or solid conductive tissue. The species of the Araceae examined had an open stylar canal. They are similar in form and structure, but have some decidedly distinguishing characteristics.

Zantedeschia aethiopica, Plate 15,Figs.1,2,has a few rows of conductive tissue lining the stylar canal. There is an abrupt transition between the conductive tissue and the adjoining parenchymatous tissue. The former being composed of narrow, regular, elongated cells with oblique cross walls while the ground tissue is composed of cells which are larger, more irregular and with much less dense content. Contrary to the observations of Behrens (1) and Capus (2) the cells of the conductive tissue are quite compact and very regularly arranged even though there are narrow intercellular spaces, especially opposite the end walls.

The cells of the conductive tissue have a dense, granular cytoplasm which is very uniformly distributed throughout the cell. The nuclei are very large and may be spherical, but more often are somewhat oval or elongated in the direction of the long axis of the cell. These nuclei are extremely large in proportion to the size of the cell. They occupy the greater part of the cavity of the cell, are very uniform in density and contain numerous very dark masses of chromatin.

The stylar canal is lined with papillae which extend into the ovary as a layer upon the placenta and extend from the tip of the style as a cluster of stigmatic papillae. These cells in the canal and ovary break down forming a dense mucilaginous substance which fills the canal aiding in the passage of the pollen tubes to the ovary. Later this mucilaginous substance fills the cavity of the ovary completely embedding the seeds.

Calla palustris, Plate 14, Fig. 1, 2, is similar to Zantedeschia aethiopica, but it has a wider stylar canal with larger papillae lining the canal and many more mucilaginous papillae on the placenta of the ovary. The cells of the conductive tissue are larger, have a less dense content, smaller nuclei, and are much more loosely arranged than those of Zantedeschia aethiopica. The Skunk Cabbage, Symplocarpus foetidus, Plate 15, Fig.3, has an open stylar canal which is very narrow and lined with a few rows of conductive tissue. These cells are longer, more loosely arranged and much less dense than those of the above two species. The cytoplasm of these cells is scant and is pushed outward about the cell wall except for strands which suspend the very large nucleus at the center of the cell, thus forming a large vacuole at each side of the nucleus. In these cells the nuclei are almost as large as the width of the cell.

The Jack in the Pulpit, Arisaema triphyllum, Plate 13, Figs. 1,2, has an open stylar canal which is densely lined with mucilaginous papillae which become very much elongated and form a brush-like mass at each end of the canal, stigmatic papillae at the outer extremity and mucilaginous cells at the base of the style. Plate 13,Fig.2.

The conductive tissue, from which the papillae arise in all these forms,lines the canal and extends over the lobes of the style to the outer edge where the papillae end and the epidermis begins. That is, the conductive tissue underlies and gives rise to the papillae whatever their position. The conductive tissue of this species is composed of long, narrow cells with dense cytoplasm, rather uniformly scattered through the cell, and large nuclei which are very much elongated.

Ismene Calathene, Plate 18, Fig. 1, has an open canal lined with conductive tissue five to eight cells in width. These cells are very loosely arranged, narrow and very much elongated. The cytoplasm is scattered throughout the cell, but is very vacuolate. The cytoplasmic strands which separate the vacuoles are very dense due to numerous dark granules. The nuclei of these cells are unusually large and much elongated, but with their ends flattened instead of pointed as in some forms. These nuclei are not uniformly dense, but are very little denser than the cytoplasm except for the dark, granular chromatin which forms irregular spirals about the nuclei.

In species with solid conductive tissue, there is rather wide variation in the amount, form and density of the tissue as well as in the shape, size and density of the nuclei of its cells.

Hibiscus Sp., Plate 19, Fig. 2, has about one-half of its style occupied by conductive tissue. This tissue widens and ends at the tip of the style in a knob-shaped enlargement, which shows a fan-shaped arrangement of the rows of cells as seen in longitudinal sections. It is covered with long papillae.

The cells of the conductive tissue are long and narrow in the lower portion of the style, but become much less regular in the expanded portion. The end walls are either oblique, in which case the cells dove-tail together, or are straight or slightly rounded. This tissue is very uniform throughout, but is delicate and loosely arranged even though it has the appearance of being compact. The cytoplasm of these cells appears very dense in comparison with the cells of the ground tissue. It is not uniformly distributed throughout the cell, but contains many small vacuoles which are separated by dense granular strands of cytoplasm. Scattered through the cytoplasm are many dark granules.

The nuclei of this tissue are of two very different types. Those which are large, spherical, and not dense, are found in the larger, more rectangular cells; while those which are elongated, often drawn out to almost needle-like points, and so dense that they stain almost black, occur in both the larger, rectangular cells and the longer, fiber-like cells. Scattered through the conductive tissue are cells with less dense cytoplasm. They are uniformly distributed throughout but are more abundant in the expanded portion of the style.

Malvaviscus Mollis, Plate 19, Fig. 1, is similar in appearance to Hibiscus sp., but in form it is more nearly club-shaped.

The cells of the conductive tissue are not so much elongated, and are more irregular than are those of Hibiscus sp. The cells of this tissue are closely arranged and appear very compact in longitudinal section, Plate 19, Fig. 2. In cross section they are round, have thick walls, and very small intercellular spaces. The cytoplasm of these cells is granular in appearance and is rather uniformly scattered throughout the cell. The nuclei are all spherical and quite dense.

Juglans cinèrea differs from the other forms observed in that its conductive tissue is not uniform. This does not agree with Behrens (1) who says that the conductive tissue is uniform throughout where it does occur. In this species this tissue is very irregular as to size and shape. There are long, irregular, rectangular cells alternating with groups of small almost square cells. The larger cells have a rather dense cytoplasm with numerous vacuoles and large nuclei which are usually somewhat elongated or oval in shape. The shorter cells are wider in the radial direction, and the cross-walls are in most cases curved, Plates 5 and 6. The cytoplasm in thse cells is dense and in most cases it has no vacuoles. The nuclei are about the size of those of the larger cells but are spherical instead of being elongated. In both types of cells are many starch grains which, however, are more numerous in the ground tissue. Juglans nigra, Plate 17, Fig. 1, differs slightly from Juglans cinèrea in the branching of the style, but they are otherwise similar.

Prunus virginian, Plate 26, Fig. 1, and Prunus sp. Plate 20, Fig. 1, are similar in that they have a large cylinder of very dense conductive tissue which is loosely arranged. Due to the density of they cytoplasm, which contains many dark granules, and the smallness of the cells it is sharply differentiated from the parenchymatous tissue which has much larger, more regular and less dense cells.

In both species the nuclei are rather small and in most cases spherical, although they may be slightly elongated, especially in Prunus sp. The conductive tissue extends down through the style into the ovary where it divides one branch going to each of the two ovules.

The tomato, Lycopersicon esculentum, Plate 16, Fig. 1, has a small style, one-third or more of which is occupied by the conductive tissue. The cells of this tissue are long and narrow with rather thick walls, but they are very loosely arranged and have large intercellular spaces. The cytoplasm of the cells is very dense due to the many dark granules scattered through it. The nuclei are nearly spherical in spite of the narrow, elongated character of the cells.

Schizanthus sp., Plate 16, Fig. 2, has conductive tissue similar to that of Lycopersicon esculentum in form, arrangement and loose character of cells, but it is peculiar in that the cytoplasm is in the form of a network which looks like a chain of beads. Its nuclei are rather small, spherical to oval in shape, and are in most cases almost obscured by the dense cytoplasm. In Phlox divaricata, Plate 24, Figs.1,2, and Plate 10, the conductive tissue is composed of elongated cells whose end walls are usually oblique so that the cells dove-tail together giving them a very compact appearance, but this tissue is very delicate and easily broken down. The cytoplasm is granular rather than reliculate in appearance, but it contains many vacuoles. The nuclei are large and very peculiar in that they are much elongated, in many cases the ends being drawn out to needle-like points, Plate 10. The chromatin is very dense, and in some nuclei appears almost as a spiral band about the nuclei. These peculiar nuclei with needle-like points are also present in the conductive tissue of Mamillaria pusilla. In this species the nuclei are sharply pointed even when they are quite large in diameter at the center.

Fuchsia sp., Plates 1,2,3,4,22,23, has conductive tissue composed of irregular, rather loosely arranged cells. Most of the cells of this tissue contain only scant cytoplasm rather uniformly distributed throught the cell, except that it is slightly denser just within the cell wall. But scattered through this tissue are cells entirely filled with a dense, brownish substance which is granular in appearance and arranged in a foamy-like mass, Plates 1,2,3,4.

Stigmatic Papillae

Behrens (1) states that the stigma is that part of they style capable of taking up pollen, but that it is not an organ entirely separable from the style, and histologically little different from it. He distinquishes between the style and stigma, which he says pass so gradually into each other it is impossible to tell where one ends and the other begins, by the fact that in all cases the style is covered with a uniform epidermis while in the stigma this is lacking or changed into a differently appearing secretion layer. He says that all stigmas show, on the outer surface, structures which are essentially secretion organs, and which are morphologically the same as the epidermis but never form a firm outer skin with a cutinized layer.

He considered the stigma to be composed of ground tissue, conductive tissue, and fibro-vascular bundle ends. These tissues both in the style and stigma are arranged in rows parallel to the long axis of the style. He designates these rows by the term longitudinal rows. At the tip he says the longitudinal rows more or less disappear, and the end cells appear free on the surface as projections or papillae forming what he terms a simple stigma of which he cited the following types.

Veronica grandis whose end walls have a thick cuticular-like membrane which secretes the stigmatic fluid.

Orobanche galii which has longitudinal rows with slanting cross walls, and weak longitudinal walls. The whole structure resembles a mass of fungal hyphae held together side by side. One or more of the end cells of the longitudinal rows may form papillae. This form of stigma he says is a very common type occurring in many plants.

Thalictrum aquilegifolium, which is a wind pollinated form is similar to Orobanche galii except that the papillae are closer together, more irregular and do not appear so distinctly as the end cells of longitudinal rows. Juglans cinerea, also wind pollinated, I found to have the same type of papillae. In such cases the longitudinal rows lose their identity at the tip of the style.

Camelina sativa has longitudinal rows on top of which are short very regular cells which carry uniform and very sharply differentiated papillae.

In Polygonum viviparum the longitudinal rows break up into an irregular mass of cells while the papillae form a very regular layer.

Myriophyllum verticillatum and Helianthemum mutabile have many-celled papillae. In such cases Behren's (1) says that the term papillae does not well apply as they are really a row or mass of cells. Capus (2) classes stigmatic papillae into simple and compound papillae. He terms simple papillae those which are formed by a certain number of epidermal cells which cover an axilary fibro-vascular bundle as in the Gramineae and Cyperaceae.

When papillae are formed of many juxtaposited cells, he calls them compound papillae as in Reseda alba, Passiflora, Corylopsis, Rubus, Sanguisorba and others.

The surface of the stigma has great variation in form, but in most cases it assumes the form of hairs or papillae. There is also great variation in the form and size of the papillae. In most cases there is but one type of papillae on one stigma, but Behrens (1) found an exception to this in Hyoscyamus niger which has knob-like to very long papillae.

The connection between the papillae and cells below also varies greatly. In Cyperaceae and Gramineae, the cells stand out along the side of the style branch. In some cases, there is an abrupt transition between the two structures as in Camelina sativa but in general Behrens (1) found that the longitudinal rows merely end as papillae without much modification as in Orobanche galii or somewhat modified as in Thalictrum aquilegifolium.

The membrane of the stigmatic papillae is uniform in most cases, but it may be swollen as in Lysimachia punctata or may be heavily cutinized. Behrens (1) notes that the tissues of the stigma are well adapted to permit the penetration of pollen tubes, for the cells are so delicate that they can be macerated with distilled water and the longitudinal rows permit the pollen tubes to crawl through them. The great advance in the development of cytological technique is brought out by the fact that he laments the fact that no reagent had yet been found that would show the penetration of pollen tubes.

In the forms observed in this investigation, a wide variation in stigmatic papillae was found. Juglans cinerea, Plate 17, Figs. 2,3, and Plate 7, Juglans nigra, Plate 17, Fig. 1, Lycopersicon esculentum, Plate 16, Fig. 1, Schizanthus sp.,

Plate 16, Fig. 2 and Ismene Calathena Plate 18, Fig. 1, have simple papillae formed from the end cells of the longitudinal rows.

Lycopersicon esculentum, Plate 16, Fig. 1, has a very loosely arranged conductive tissue which becomes still looser near the tip of the style thus forming a slightly enlarged tip in which the end cells of the conductive tissue enlarge slightly forming the papillae. Except for this slight enlargement, the papillae differ little from the cells of the conductive tissue in the style which has been described previously. In the receptive stage the cells of the stigmatic area are embedded in a foamy mass of stigmatic fluid.

Schizanthus sp., Plate 16, Fig. 2, has its stigmatic area formed in practically the same manner as Lycopersicon esculentum except that the conductive tissue instead of protruding or overflowing at the tip of the style merely comes to its surface loosely occupying this region. The papillae are similar to the cells of the conductive tissue except that they are slightly enlarged at the tip. Their cytoplasm is quite dense and contains many dark staining granules. The nuclei are small and lie near the tip of the papillae.

Although the papillae of Juglans cinerea, Plate 17, Figs. 2,3, are merely modifications of the terminal cells of the longitudinal rows of conductive tissue, like those of Thalictrum aquilegifolium, Behrens (1), they do not appear distinctly as such. These papillae are swollen and rounded at the tip. The cytoplasm is not dense and lines the wall with an irregular layer leaving the greater part of the cell occupied by a large vacuole.

The nuclei of these cells are large and lie embedded in the cytoplasm at, or near, the tip where the cell content is the denest, Plate 7. Juglans nigra, Plate 17, Fig. 1, has practically the same structure except that the papillae in this species are more regular forming a uniform layer.

Other forms studied show a very definite line of differentiation, due to differences in size, structure, density, etc., between the papillae and the conductive tissue of the style.

Malvaviscus Mollis and Hibiscus sp. are similar. Plate 19, Figs. 1,2. They have their conductive tissue arranged in longitudinal rows which lose somewhat their regularity in the enlarged portion of the style branch, due to the enlargement of the cells in width. The conductive tissue ends in a very uniform line. The papillae are wider than the cells beneath them.

The papillae are large and cone-shaped. The base of these papillae usually contains a large vacuole but the upper part of the cell is filled with a dense granular cytoplasm. The nuclei are rather large, and round or slightly flattened. They are very uniformly granular, quite dense and contain one unusually large nucleolus. They lie nearer at the point where the papillae begin to taper noticeably. The papillae of Malvaviscus Mollis are longer than those of Hibiscus sp. and contain a dense substance which appears to be oil globules.

Aquilegia canadensis has an unusual arrangement of the papillae in that they line one side of the style branch. They are disposed irregularly along this side of the branch and stand out from the surface in a brush-like mass. They arise from the conductive tissue which lies directly beneath and whose cells bend outward toward the base of the papillae. Their line of union is therefore not sharply differentiated. The papillae are somewhat oval in shape and are rather uniformly filled with cytoplasm which is very dense due to the presence of many dark granules. The nuclei are very small and lie near the center of the cell.

In Phlox divaricata, Plate 24, Figs.1,2, the style divides into two narrow branches completely covered with papillae which give to them a brush-like appearance. These cells have large swollen bases and narrow toward the tip which has a very thick layer of cuticle similar to that of Lysimachia punctata, observed by Behrens (1). These papillae are sharply differented from the cells beneath which have their longitudinal axis in the opposite direction. The cytoplasm lines the cell wall, and strands run all throught the cavity enclosing large and small vacuoles; the latter being very abundant about the nucleus giving a foamy appearance. The nuclei are large, and spherical and lie near the center of the cell, Plate 11. The Araceae studied all have an open stylar canal lined with papillae which break down to form a mucilaginous substance. At the tip of the style these papillae elongate into a brush-like cluster of stigmatic papillae.

Arisaema triphyllum, Plate 13, Figs.1,2, has long strap-shaped papillae with vacuolate cytoplasm and large spherical or slightly flattened nuclei which lie near the center of the cell. The cells of the conductive tissue lying below the papillae are very small and elongated in the opposite direction from papillae, thus making a sharp line of differentiation between these two tissues.

Symplocarpus foetidus, Plate 15, Fig. 3, has a much narrower canal than Arisaema triphyllum. In the canal the papillae are small and oval while at the tip of the style the stigmatic papillae are long and hair-like. The canal in the lower part of the style is very narrow and does not have papillae. Near the top of the style the canal widens and contains smaller papillae than those of the stigma. The stigmatic cells are very easily distinguished from those below by the fact that they are very much larger, loosely arranged and have less dense cytoplasm.

Calla palustris, Plate 14, Fig. 1,2,has one celled papillae which are much swollen or club-shaped at the tip while Zantedeschia aethiopica, Plate 15, Figs. 1,2, has several celled papillae. (Behrens (1) states that term papillae does not apply here) The cells are elongated and rectangular, and are arranged end to end in rows which lie side by side. The end cell of each papillae or row of cells is swollen at the tip and shaped much like the one-celled papillae of Calla palustris. The cells of the papillae are quite uniformly filled with a vacuolate cytoplasm. The nuclei which lie near the center of the cells are large and spherical. They contain a large dark nucleolus and densely staining chromatin. The tip cell has less dense cytoplasm which is most dense just within the cell wall. In these cells the nuclei are usually midway between the end walls but laterally placed.

In Fuchsia, sp., Plates 2,3, Fig.1 and Plate 22, Fig.4, the style ends in a swollen four-lobed structure which is covered with large, irregular and closely massed stigmatic papillae. The cytoplasm of these cells is rather dense especially at the tip of the cells. The cells may have several small vacuolus or one very large one which pushes the cytoplasm out about the walls. The nuclei are large and lie midway between the ends of the cells laterally placed. These nuclei are not dense except for the numerous chromatin masses which stain very dark.

In the receptive stage of the stigma a reticulate substance fills the cavity between the lobes and forms a thick layer about the surface papillae. This substance is a foamy mass which is almost cell-like in appearance. Scattered through this material are many nuclear-like bodies which vary greatly in size. They may be found singly but more often are in groups of from two to five. The fact that this substance covers the stigmatic papillae indicates that it is the stigmatic fluid but the nature of nuclear-like bodies scattered through it remains for the present obscure. Plate 3.

The papillae of Prunus sp. Plate 27, and Plate 20, Fig. 1, and Prunus virginiana, Plate 26, Fig.1, are similar. The dense, elongated cells of the conductive tissue are sharply differentiated from the stigmatic papillae which are much larger, and have scant cytoplasm and conspicuous vacuoles. The cytoplasm of the papillae is mostly located at the tip of the cells. The nuclei of thse cells are small and lie near the tips . Dorsey (4) says that these cells at the receptive stage are very irregular in outline, are collapsed and shrunken, and that the cell walls appear to be broken. He found that the cytoplasm is much contracted and that the nuclei are irregular in outline and show signs of disintegration. The stigmatic fluid forms a dense foamy layer above these cells and in it are embedded pollen grains.

Ribes sp., Plate 21, Figs.1,2, and Native wild crab, Plate 20, Fig. 2 and Plate 28, Fig. 1, are in the transition stage. That is the fusion of the carpels is not complete. In Ribes, the style branches are partially united but each still retains all the tissues present in the separate styles. The conductive tissue, which occupies the greater portion of the style, ends in stigmatic papillae which are not sharply set off from the cells beneath it. They break down in the receptive stage to form a very thick, foamy mass of stigmatic fluid.

In Malus ioensis, Plate, 20, Fig. 2 and Plate 28, Fig. 1, the fusion of neither the ovaries nor styles is completely fused. The epidermal tissue extends to the center of the style thus clearly marking the line of union. Each lobe of the style still contains all the tissues of the free styles in the same position. The fusion is more complete in the style than it is in the ovary.

The uniform conductive tissue is sharply set off from the stigmatic papillae. The cells of the conductive tissue are small while the stigmatic papillae are larger and club-shaped. They are thinly scattered over the tip of the style. The papillae have a scant layer of cytoplasm just within the cell wall but the cell is completely filled with a dense brown substance. The nuclei are small and laterally placed about equal distant from the end walls. The walls of these cells are covered with a thick layer of cuticle which is especially thick at the tip of the papillae.

Summary.

1. The principal contributions to the structure of style and stigma were published by Behrens (1) 1875, and Capus, (2), 1878. They described these structures for numerous species,

but dealt chiefly with the anatomy of these structures.

2. The style is composed of epidermis, ground tissue, in which the fibro-vascular bundles are embedded, and conductive tissue. It is usually cylindrical, but may be flattened, angular etc.

3. The stigma is merely a continuation of the conductive tissue from the style. By most Botanists the term, stigma, applies to only those cells which are differentiated into papillae.

4. The various tissues which compose the style have in general the following characters:

a. Epidermis.

It is one layer of cells in thickness in all species studied, and is almost always differentiated from the cells beneath. The cells are usually regular and rectangular in shape but in some cases may be round, elliptical etc. The outer cell walls are always cuticularized. This layer is usually thin, but may be thick and irregular. The cells of the epidermis may be modified to form one-celled to many-celled hairs which may be glandular.

b. Ground tissue.

This tissue is usually composed of slightly irregular, rounded cells with thin walls, and intercellular spaces. The content of these cells is less dense than that of the conductive tissue.

c. Conductive tissue.

This tissue may line the open canal which occupies the center of the style, or itself form a solid cylinder at the center of the style. It is usually composed of elongated loosely arranged cells. The content of these cells varies in density but is denser than that of the cells in the ground tissue. The longitudinal walls are easily separated or broken down, while the cross walls are more firmly joined. This feature affords the pollen tubes an easy passage on their way to the ovary. It is this tissue that forms the path for the pollen tubes to reach the ovary.

5. The stigma is formed by the modification of terminal cells of the conductive tissue to form papillae. These papillae are more often one-celled, but may be composed of more than one cell. They vary in shape from almost round, to long cone-like cells.

6. The cells of both the style and stigma may contain, starch, tannin, mucilage, etc.

7. The content of the cells varies greatly both in the same and different tissues. The cytoplasm may be scant, reticulate and very vacuolate or dense, granular and completely filling the cell cavity. The nuclei vary in shape from spherical to much elongated. In a few forms the ends were drawn out to needle-like points. In the tissues of the style the nuclei are usually near the center of the cell, but in the papillae they are in almost all cases nearer the tip of the cell.

Literature Cited

(1) Behrens, Wilhelm Julius. Untersuchungen über den anatomischen Bau des Griffels und der Narbe einiger Pflanzenarten. Göttingen 1875

(2) Capus, M.G. Anatomie du tissu conducteur. Annales des Sciences Naturelles VI, 7 Paris 1878, pp. 209-291.

(3) Coulter, J.M. and Chamberlain, C.J. Morphology of Angiosperms.

(4) Dorsey, M.J. Relation of Weather to Fruitfulness in the Plum. Journal of Agriculture Research. Vol.XVII, No.3. Washington,D.C., June 16, 1919.

(5) Le Maout, Em. and Decaisne, Jh. Traité General de Botanique.

(6) Payer, Traité, Traiteé d'organogenie comparée de la fleur, 1857, p.737.

List of Plants Studied

Arisaèma triphullum

Calla palustris

Symplocarpus foetidus

Zantedeschia aethiopica

Fuchsia sp.

Pelargonium sp.

Mamillaria pusilla

Juglans cinèrea

Juglans nigra

Hibiscus sp.

Malvaviscus Mollis

Aquilègia canadénsis

Agapanthus umbellatus

Prunus virginiana

Prunus sp.

Phlox divaricata

Lycopersicon esculentum

Schizanthus sp.

Ismene Calathene

Ribes sp.

Tropaeolum sp.

Curcurbita sativus

Salvia sp.

Primula sp.

Cereus flagelliformis

Plumbago capensis

Gloxinia sp.

Hippeastrum Johnsoni

Hymenocallis speciosa

Nerine erubescens

Ipomaea

Antirrhinum sp.

Swainsonia galegifolia albiflora

Thunbergia alata

Clerodendron tompsonae

Cleome sp.

Malus ioensis

Malus sylvestris

Description of Plates

All figures were drawn with the aid of a camera lucida, Bausch and Lomb ocular, and objectives 16 mm. 4 mm. and oil immersion 1.9.

The outline drawing, Plates. I, was made with the 16 mm. objective, the outline drawing, Plate V, with the 4 mm. objective, and all drawings showing cellular detail, Plates II, III, IV, VI, VII, VIII, IX, X, XI, XII, with the oil immersion 1.9 mm. objective.

The photomicrographs, Plates XIII to XXIX inclusive, were made with a Bausch and Lomb horizontal photomicrographic machine using a Leitz No.2, and Bausch and Lomb 16 mm. and 4 mm. objectives, and a Zeiss No. 2 compens. ocular. The figures are all from longitudinal sections unless otherwise stated.

Plate I.

A group of cells from the conductive tissue in the style of Fuchsia sp.

Plate II.

A group of stigmatic papillae at the left, and the stigmatic fluid, to the right, of, Fuchsia sp.

Plate III.

A portion of the stigmatic fluid between the lobes of the stigma of Fuchsia sp., showing the nuclear-like bodies.

Plate IV.

A portion of one cell from the conductive tissue in the style of Fuchsia sp., showing the dense granular cell content.

Plate V.

An outline drawing of the conductive tissue in the style of Juglans cinerea showing the groups of small box-like cells scattered among the larger, elongated cells.

Plate VI

A drawing showing the cellular detail of the two types of cells explained in Plate V.

Plate VII.

A group of cells from the tip of a style branch of Juglans cinerea, showing the cellular detail of the stigmatic papillae, and the cells of the conductive tissue directly beneath the papillae.

Plate VIII.

A group of cells of the conductive tissue in the style of Prunus virginiana, showing the cellular detail of three cells.

Plate IX.

A group of cells from Prunus virginiana showing the cellular structure of the papillae and the outline of the cells of the conductive tissue directly beneath the papillae.

Plate X.

A group of cells from the conductive tissue of Phlox divaricata showing the cellular structure. The elongated nuclei are worthy of note.

Plate XI.

The stigmatic papillae of Phlox divaricata showing cellular structure.

Plate XII.

Fig. 1. A group of cells from the compound papillae of Zantedeschia aethiopica. This group of cells is situated about midway between the narrow cells of the conductive tissue in the style and the outermost cell of the papilla.

Fig. 2. The terminal cell of a stigmatic papilla of Zantedeschia aethiopica.

Fig. 3. A single cell, from just below the papillae, of the conductive tissue of Zanthedeschia aethiopica.

Plate XIII.

Fig. 1. Section through the style of Arisaenia triphyllum showing the stigmatic papillae.

Fig. 2. Section through the pistil of Arisaenia triphyllum showing the stylar canal lined with papillae which protrude at each end of the canal.

Plate XIV.

Fig. 1. Section of Calla palustris showing the stigmatic papillae.

Fig. 2. Section of Calla palustris showing the stylar canal with papillae and mucilaginous secretion in and at the base of the canal.

Plate XV.

Figs. 1 and 2. Sections through the style of Zantedeschia aethiopica showing the stylar canal and papillae.

Fig. 3. Section through the style of Symplocarpus foetidus

showing the stylar canal which is lined with papillae only near the tip.

Plate XVI.

Fig. 1. Section of the style of Lycopersicon esculentum showing the conductive tissue and stigmatic papillae.

Fig. 2. Section through the style of Schizanthus sp.

Plate XVII.

Fig. 1. A portion of one of the style branches of Juglans nigra.

Fig. 2. A portion of the style of Juglans cinerea showing the base of the style branches.

Fig. 3. A few lobes of a style branch showing the cellular arrangement.

Plate XVIII.

Fig. 1. The upper portion of the style, and the loosely arranged papillae of Ismene Calathene.

Fig. 2. The upper portion of the style of Agapanthus umbellatus showing the loose nature of the conductive tissue and the stigmatic papillae.

Plate XIX.

Fig. 1. Upper portion of the style of Malvaviscus Mollis showing the nature of the conductive tissue and papillae.

Fig. 2. Upper portion of the style of Hibiscus sp.

Plate XX.

Fig. 1. Upper portion of the style of Prunus sp. showing the conductive tissue spreading over the tip of the style and covered with a layer of stigmatic fluid in which are embedded pollen grains.

Plate XX.

Fig. 2. The tip of a style branch of _Malus ioensis_ showing the tissues of style and stigmatic papillae with their heavily cutinized walls.

Plate XXI.

Figs. 1 and 2. A portion of the style of _Ribes sp._ showing the partially fused styles, each with its own tissues. The stigmatic papillae are broken and surrounded by the stigmatic fluid in which are embedded many pollen grains.

Plate XXII.

Fig. 1. A portion of the conductive tissue from the style of _Fuchsia sp._ showing a portion of a cell filled with a brown, granular substance.

Fig. 2. The same structure described in Plate XXII, Fig. 1, but less magnified.

Fig. 3. The region between the lobes of the stigma of _Fuchsia sp._ showing the dense stigmatic fluid between these lobes.

Fig. 4. Section through the lobes of the stigma of _Fuchsia sp._ showing the layer of papillae surrounding each lobe.

Plate XXIII.

Fig. 1. A cross section of the lobes of the stigma of _Fuchsia sp._ showing the lobes surrounded with papillae about which is the stigmatic fluid.

Fig. 2. A cross section of the stigma of _Fuchsia sp._ just below the union of the lobes.

Plate XXIV.

Figs.1 and 2. Style branches of <u>Phlox divaricata</u>. The papillae surround the style branches. The entire style branches are not entire in these sections.

Plate XXV.

Fig. 1. A cavity in the style of <u>Aquilegia canadensis</u> in which is found a mass of dark staining granular material.

Fig. 2. Style branch of <u>Mamillaria pusilla</u> showing the irregular arrangement of the papillae.

Plate XXVI.

Fig. 1. The style of <u>Prunus virginiana</u> showing the distinct differentiation between the epidermis, ground tissue, conductive tissue, and papillae.

Fig. a. A single style branch of <u>Pelargonium sp</u>. showing the sharp differentiation between the tissues. The papillae line only one side of the style branch, and they are multicellular.

Plate XXVII.

Figs.1 and 2 from "A Study of Sterility in the Plum." J.M.Dorsey (1919).

Figs. 3 to 8 inclusive from "Relation of Weather to Fruitfulness in the Plum." J.M.Dorsey (1919).

Figs.1,2 and 3. Stigmas of <u>Prunus</u>, Minnesota No.21, showing the tissues of the stigma and style, pollen grains in the stigmatic fluid and pollen tubes growing through the conductive tissue.

Fig. 4. Stigma of Prunus, Minnesota, No.35 showing the broken down condition of the conductive tissue, and the dense stigmatic fluid.

Fig. 5. Stigma of Prunus, Sapa before it is receptive showing clearly the nature of the papillae.

Fig. 6. Stigma of Prunus, Opata before the receptive stage.

Fig. 7. Style of Prunus, Minnesota No.35 showing the abscission layer.

Fig. 8. The surface of the abscission layer of Prunus, Assiniboin after the style has fallen.

Plate XXVIII.

Fig. 1. The cross section of Malus ioensis showing the partially fused styles.

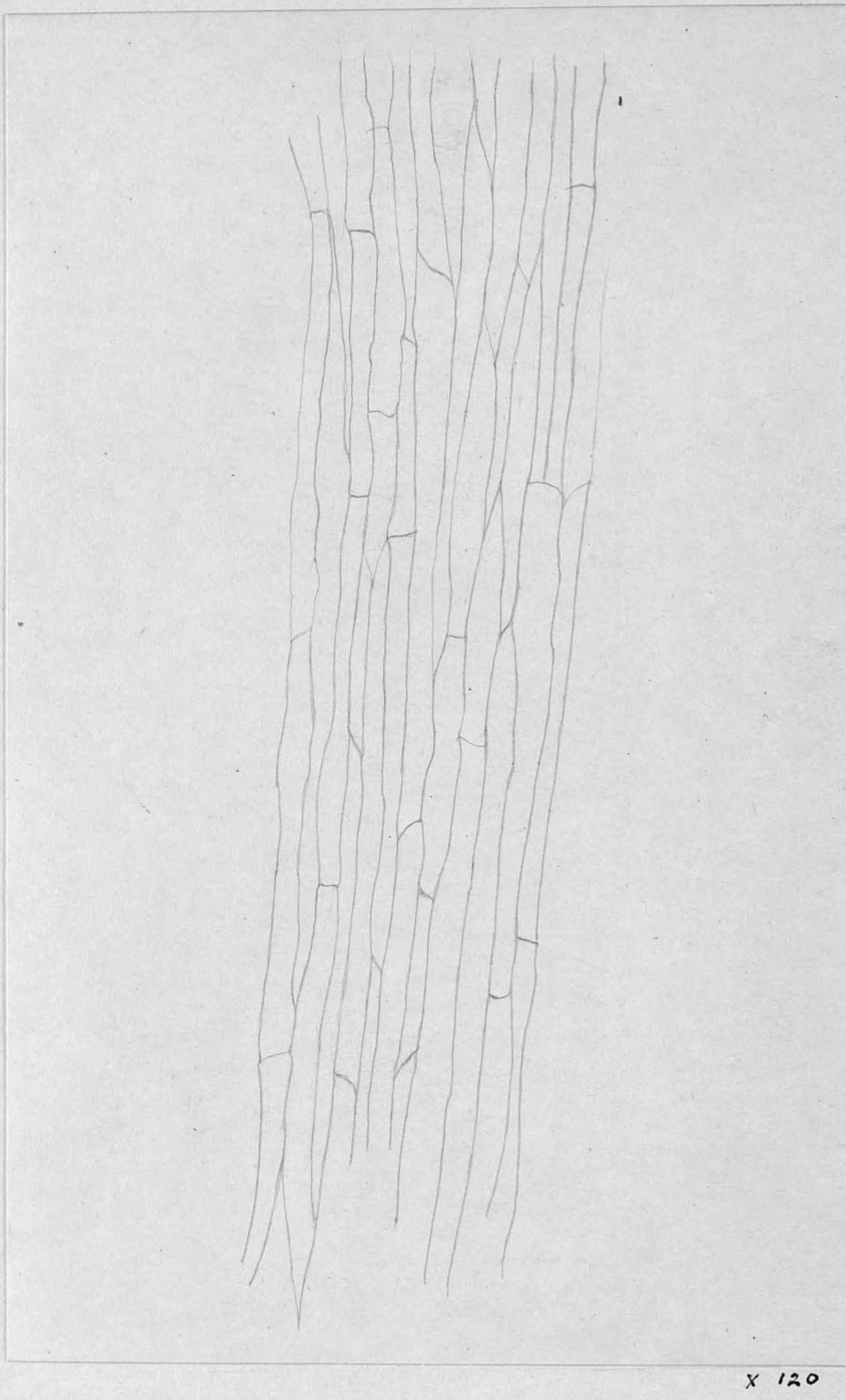
Fig. 2. Cross section of the style of Juglans cinerea at the point where the style begins to branch. A vascular strand passes into each lobe of the style branches.

Plate XXIX.

Fig. 1. A cross section of the style of Hibiscus sp. showing the arrangement of its tissues.

Fig. 2. A cross section through the style of Fuchsia sp. showing the four fibro-vascular bundles corresponding to the four lobes of the stigma.

Plate I.



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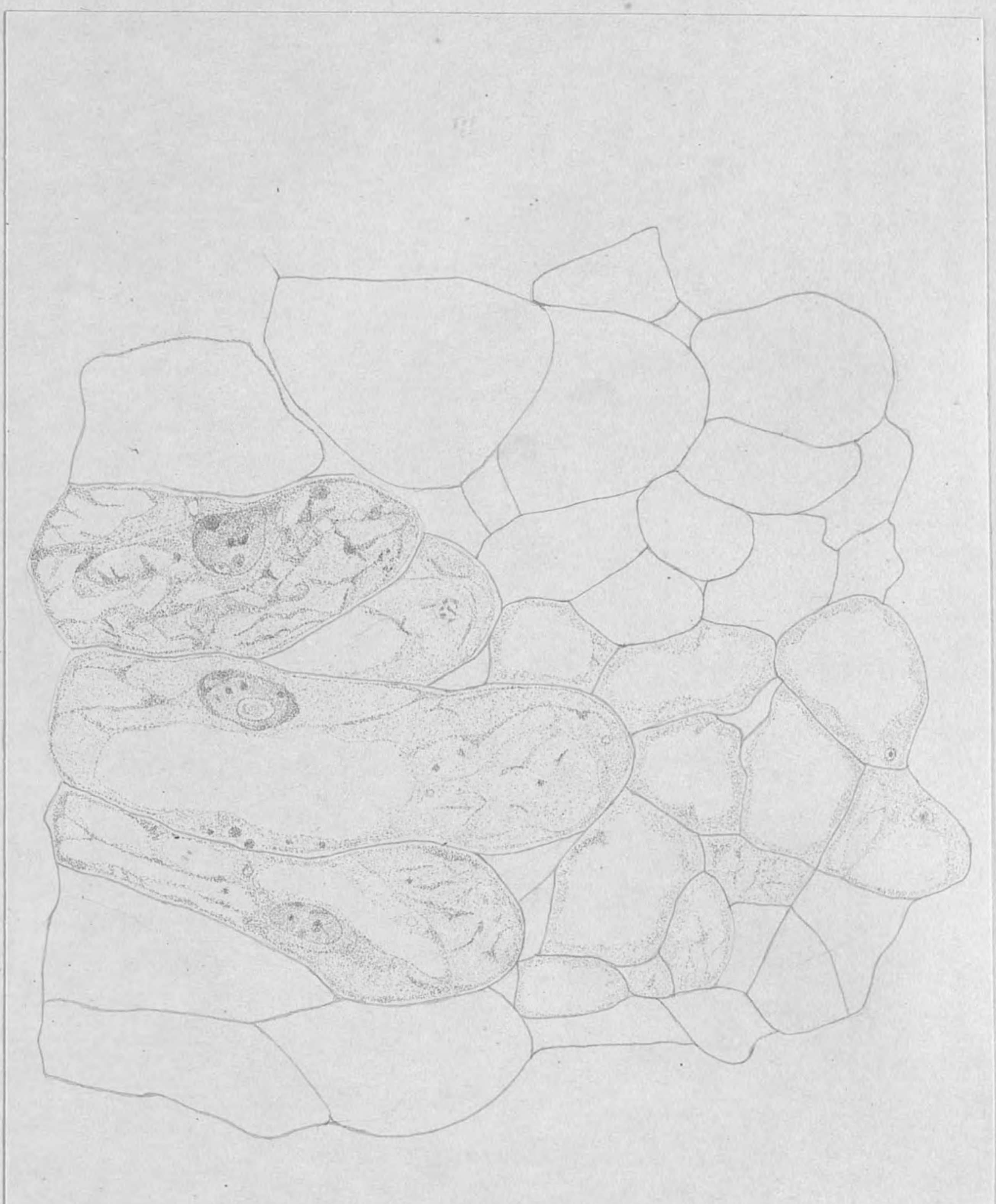

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Plate III.

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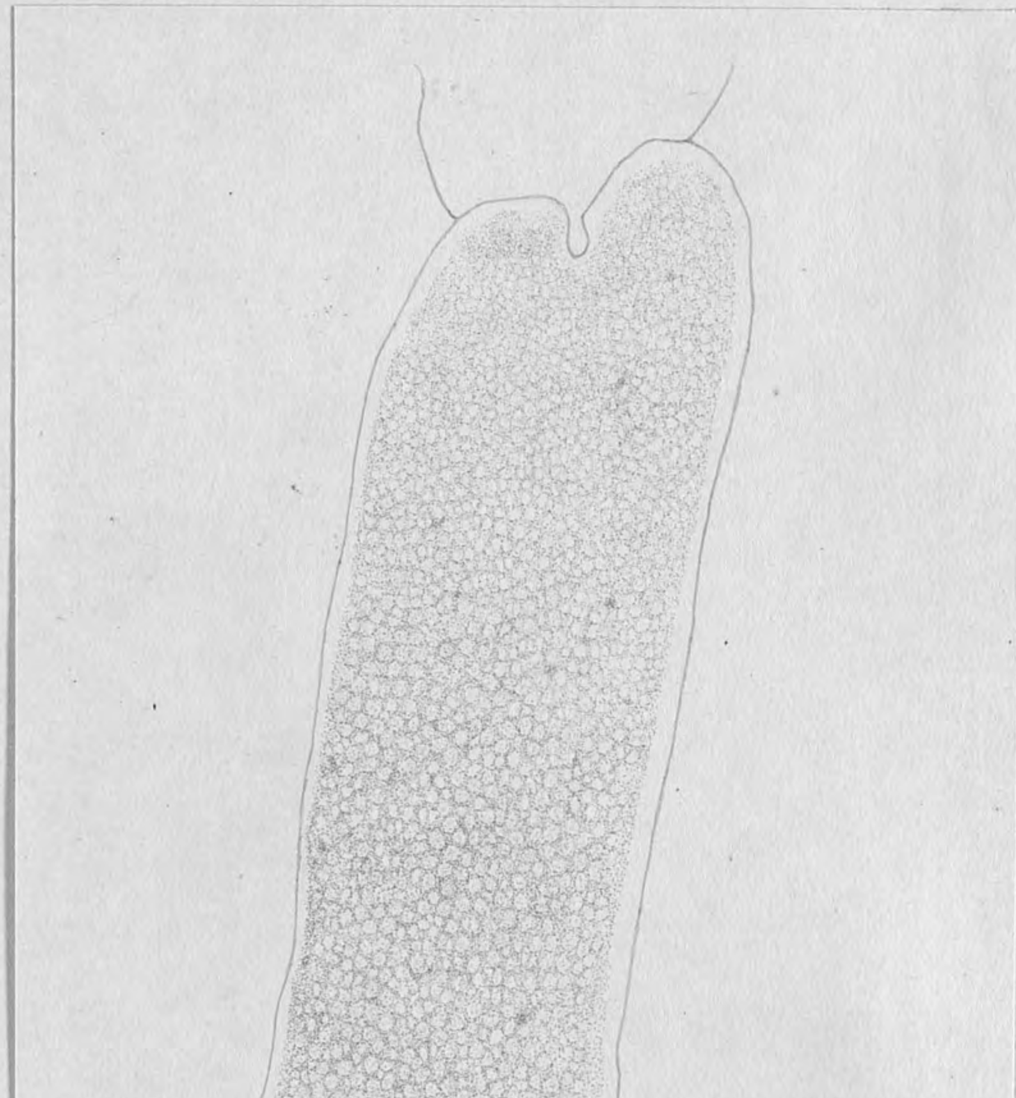
Plate IV.



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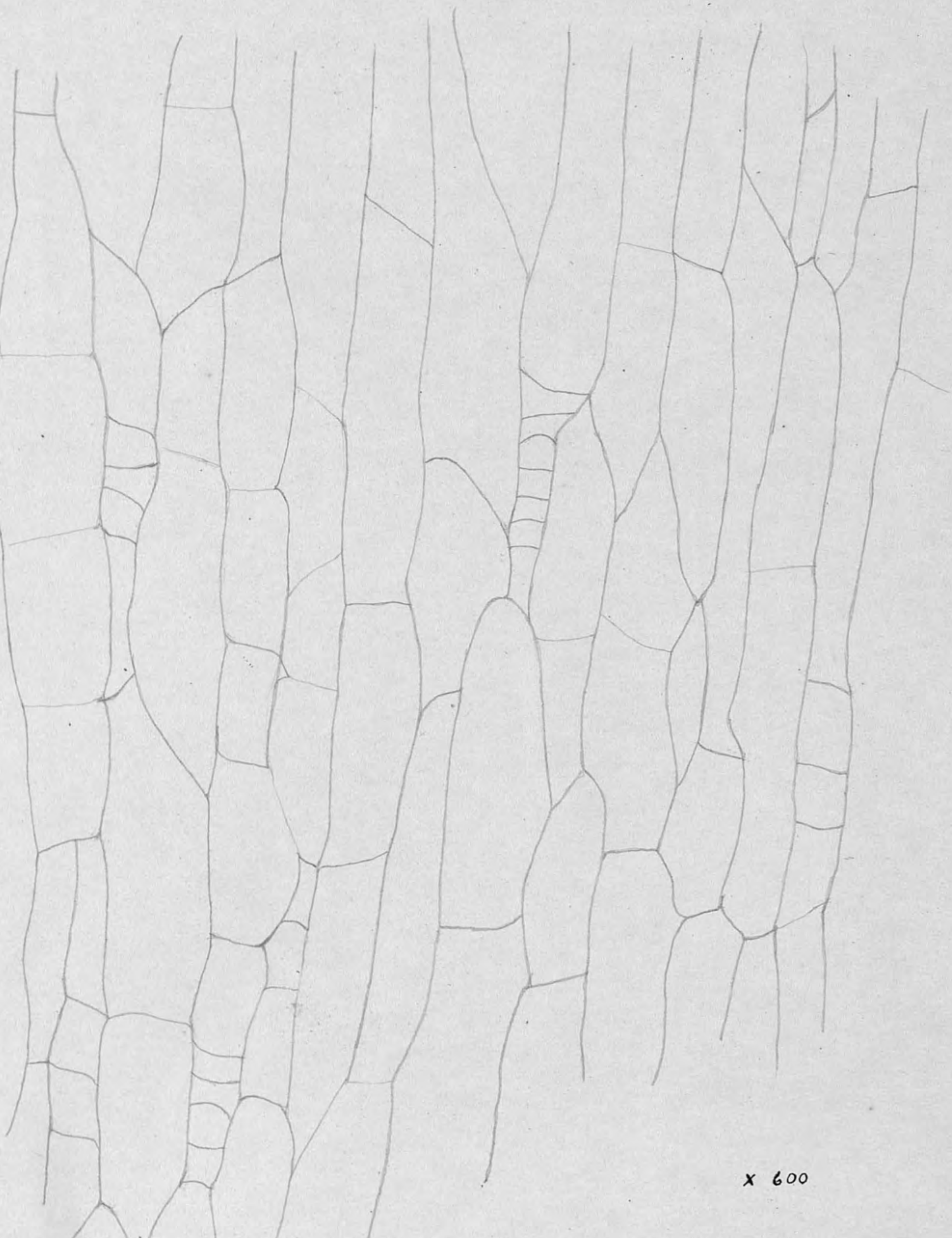

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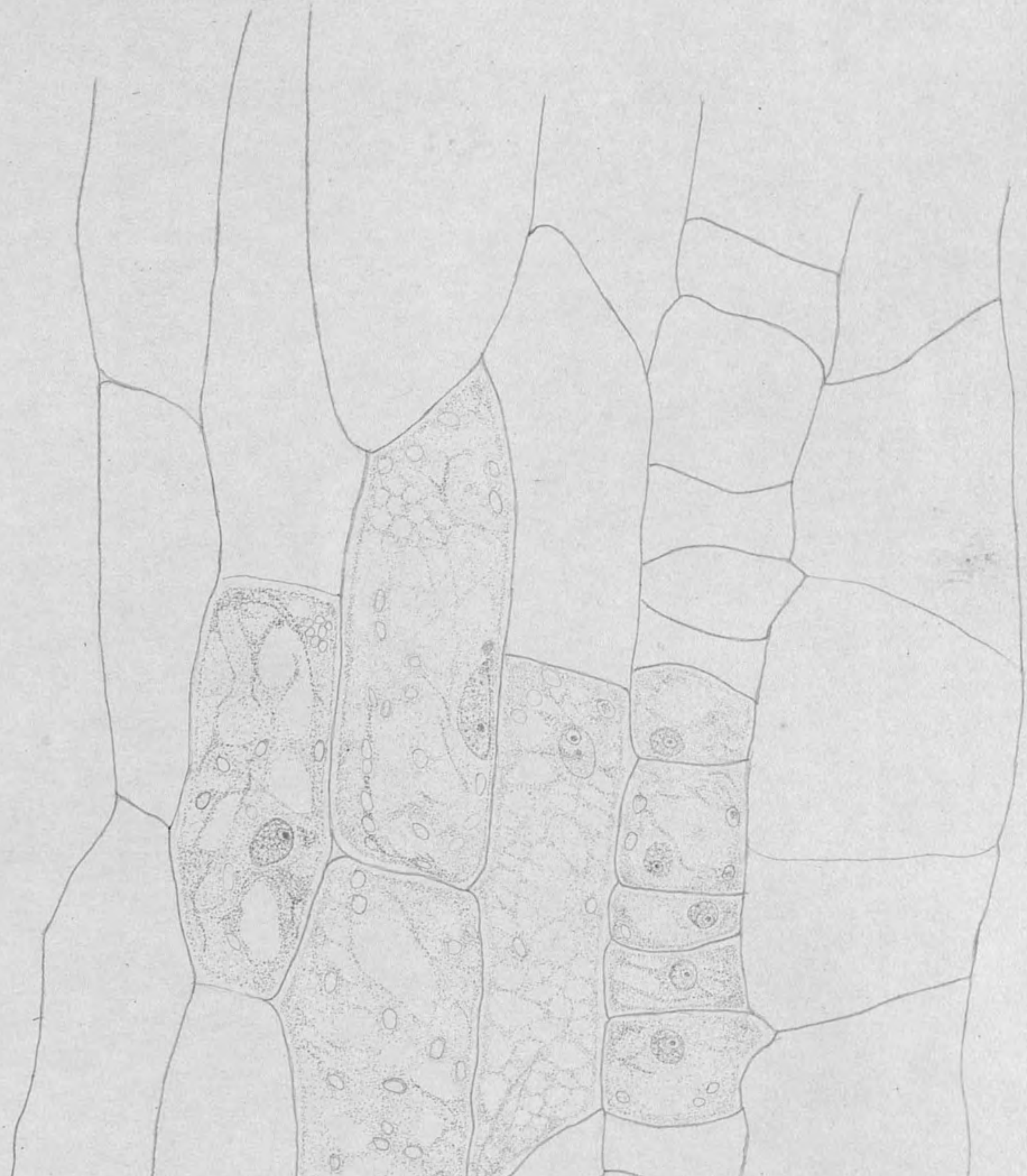

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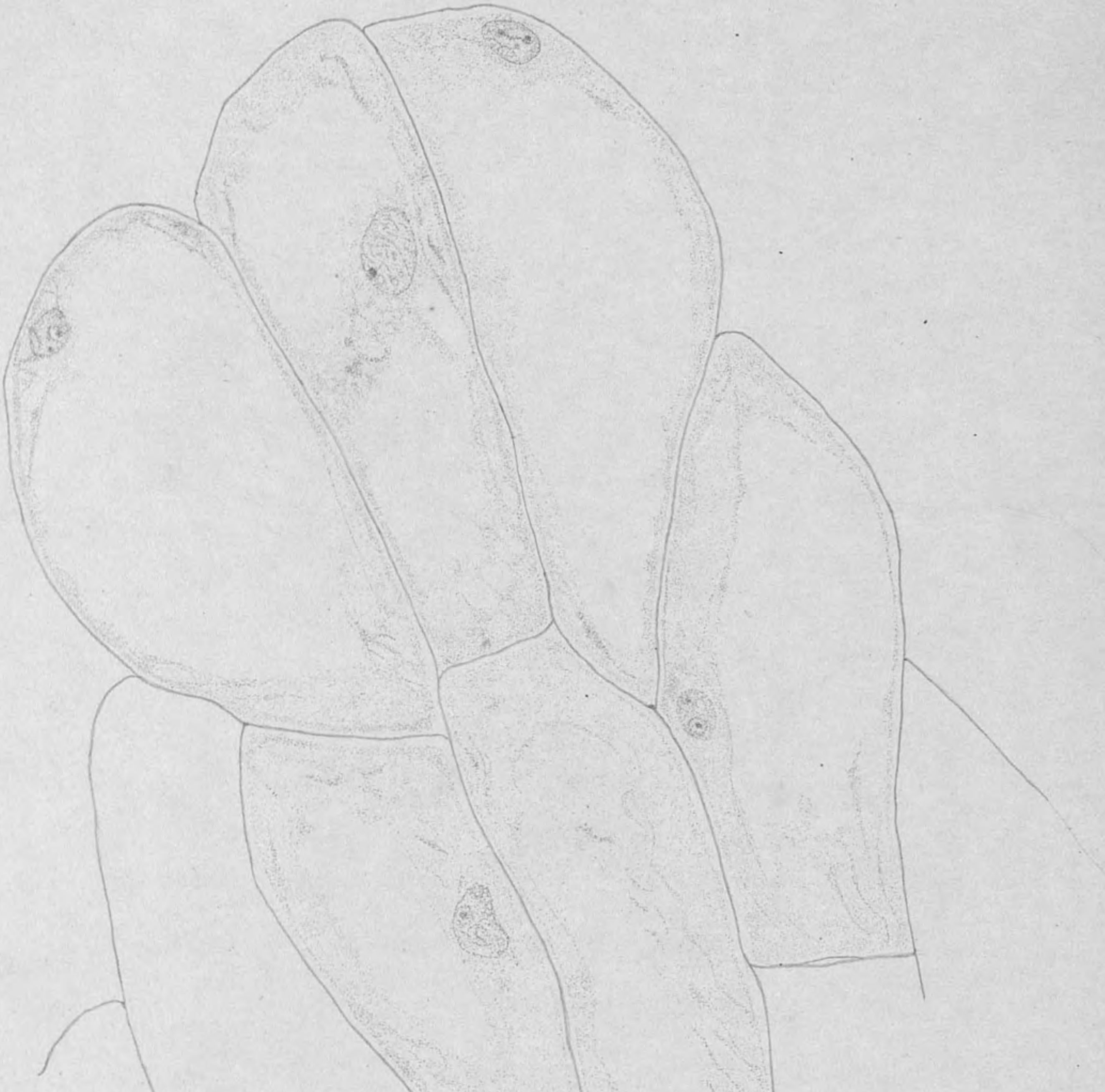

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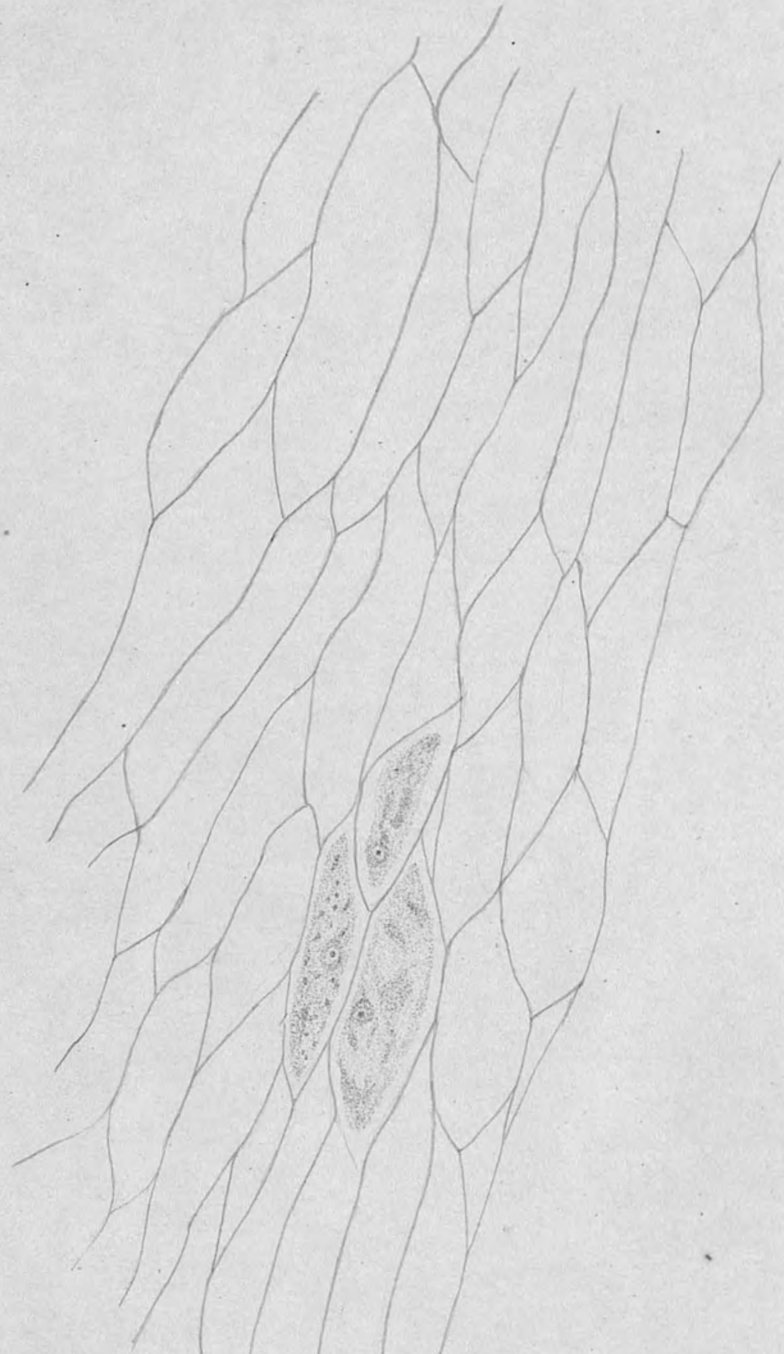

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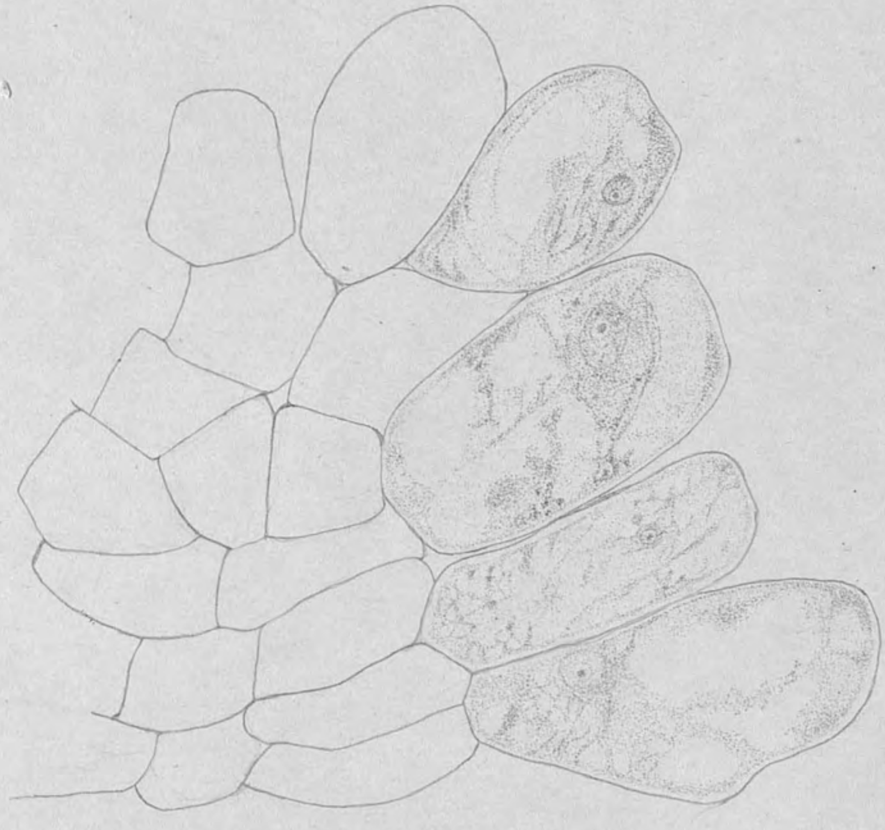

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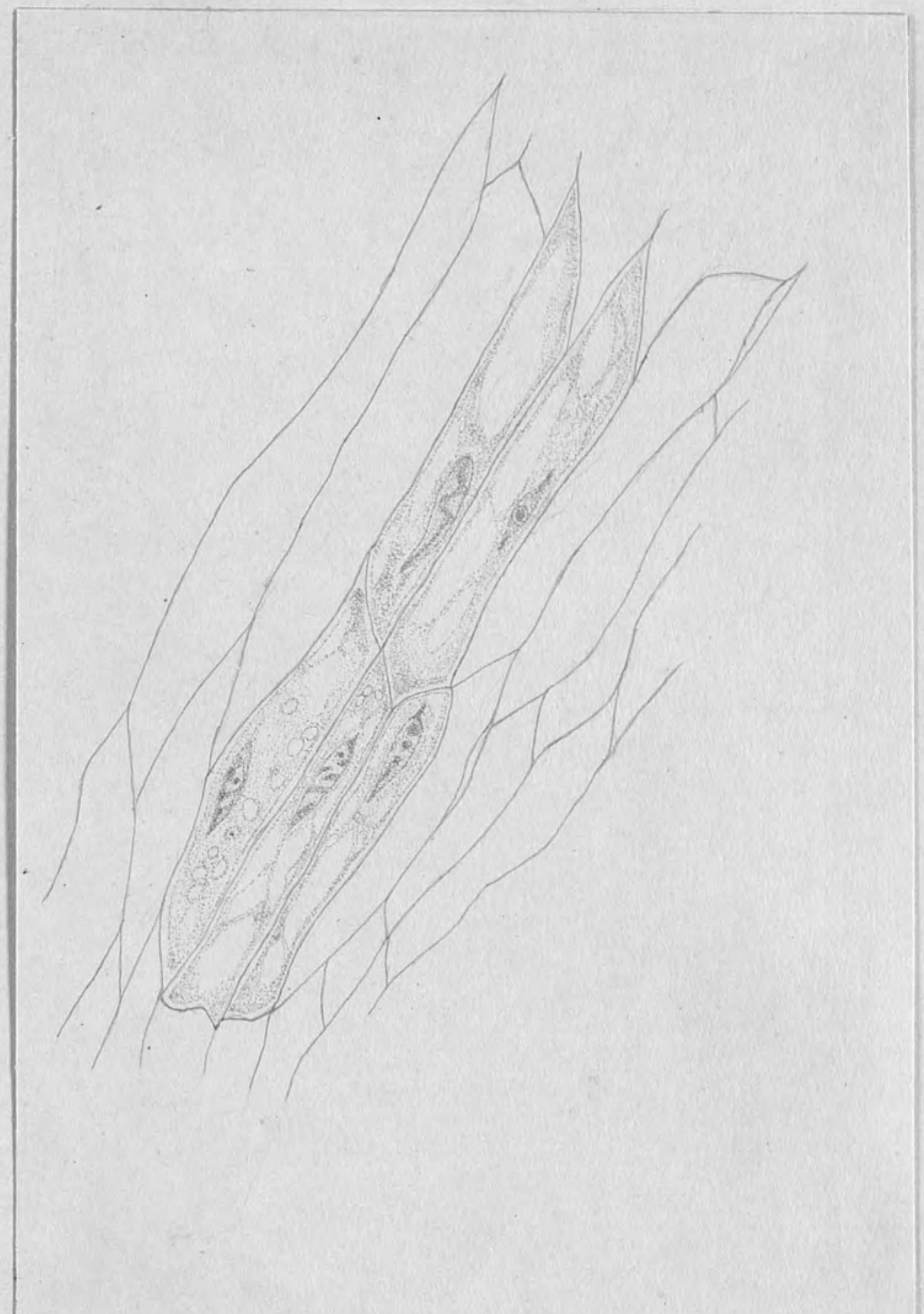

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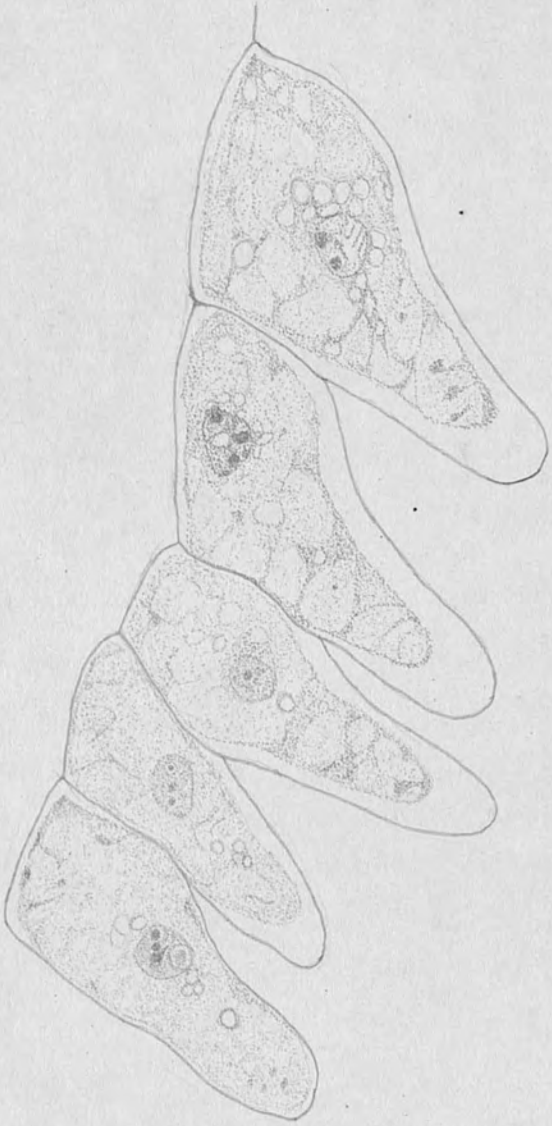

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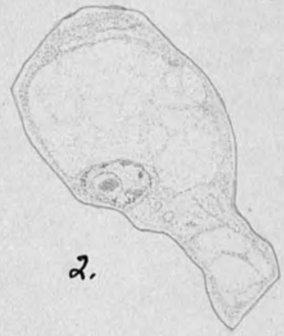

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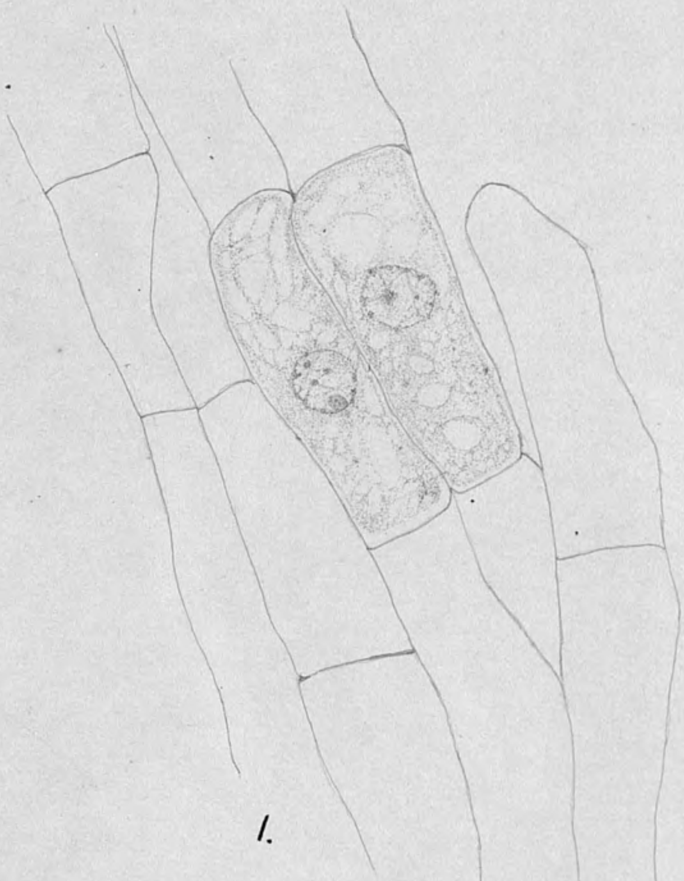

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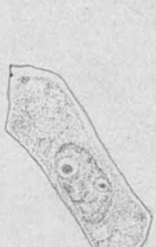

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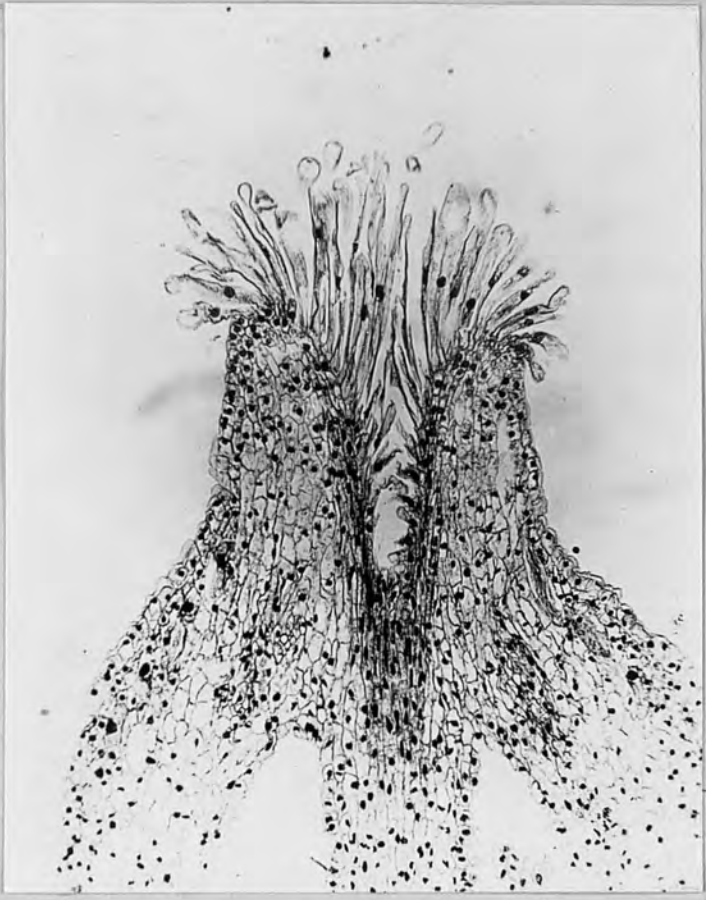

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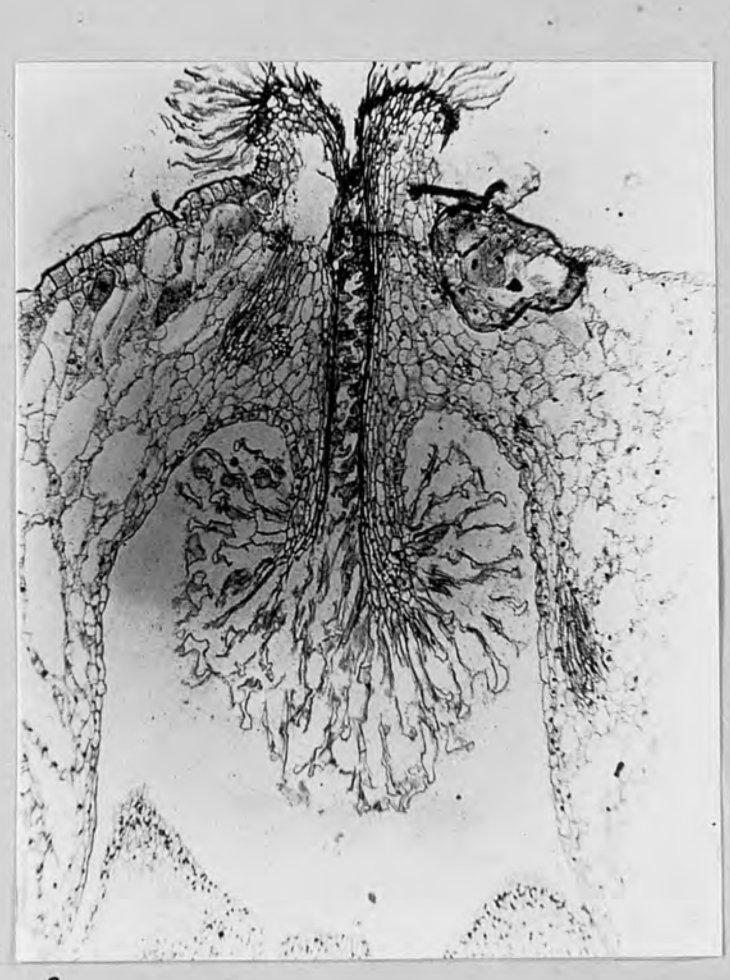

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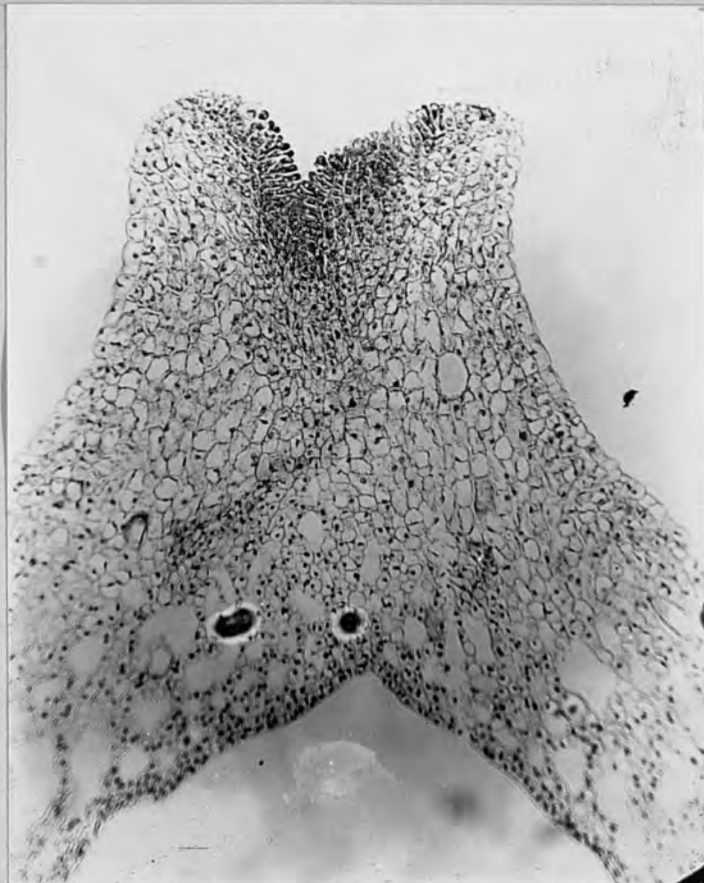

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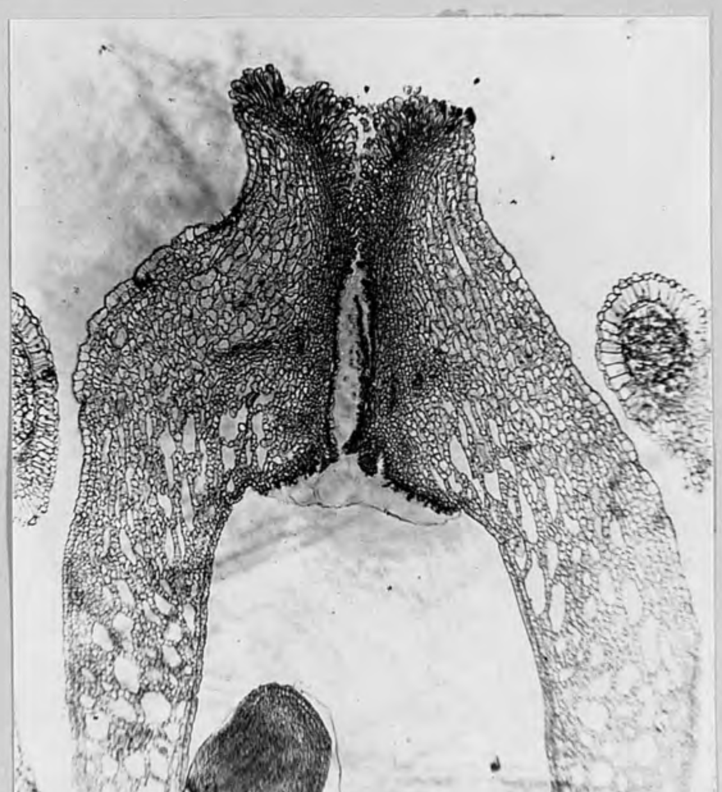

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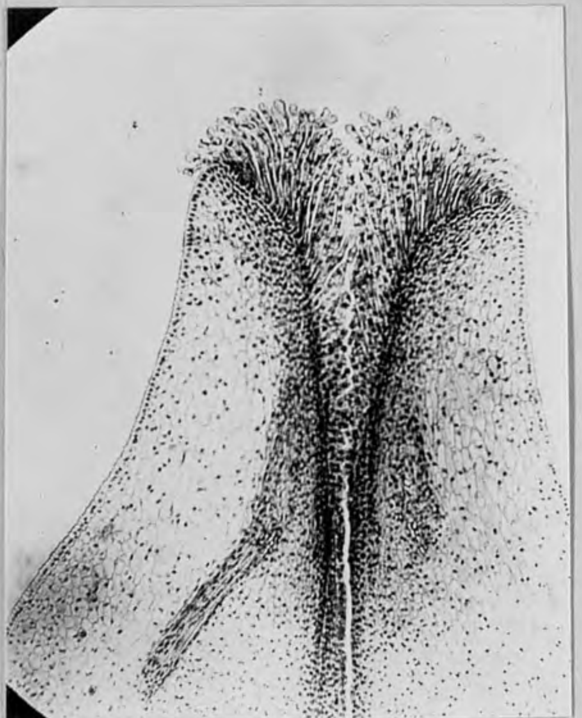

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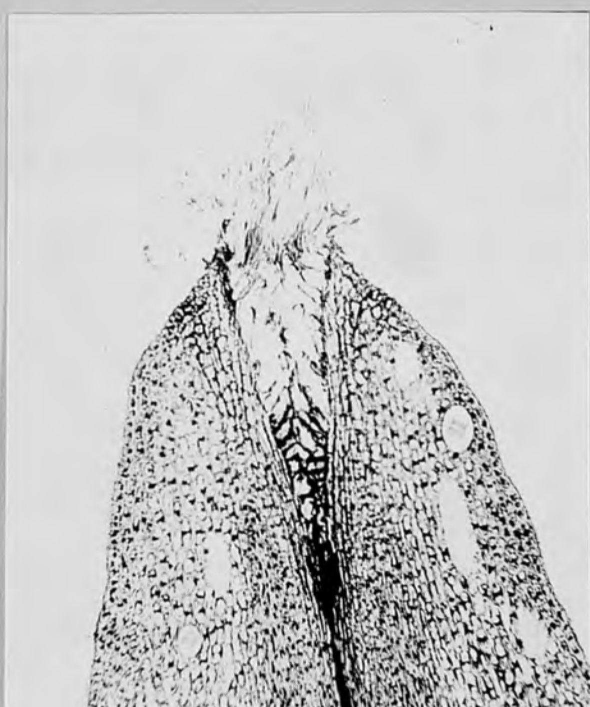

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Plate XVI.

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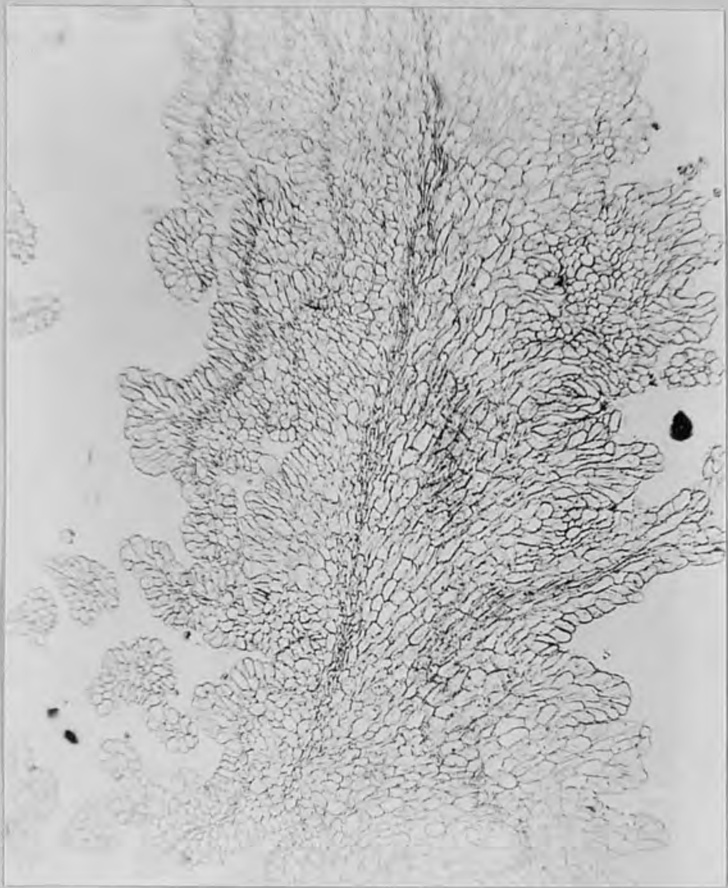
Plate XVII.



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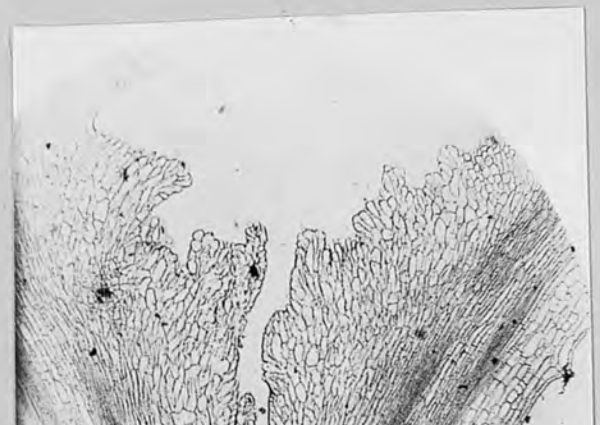

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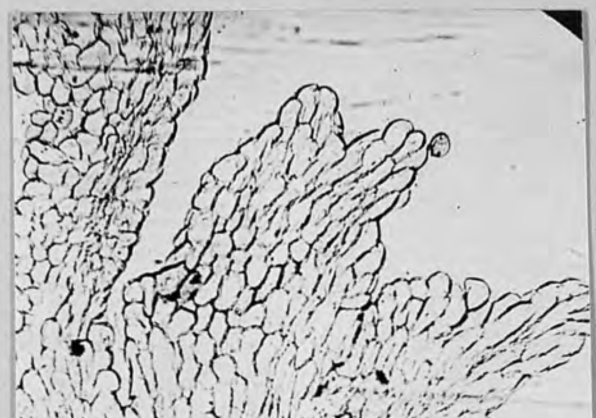

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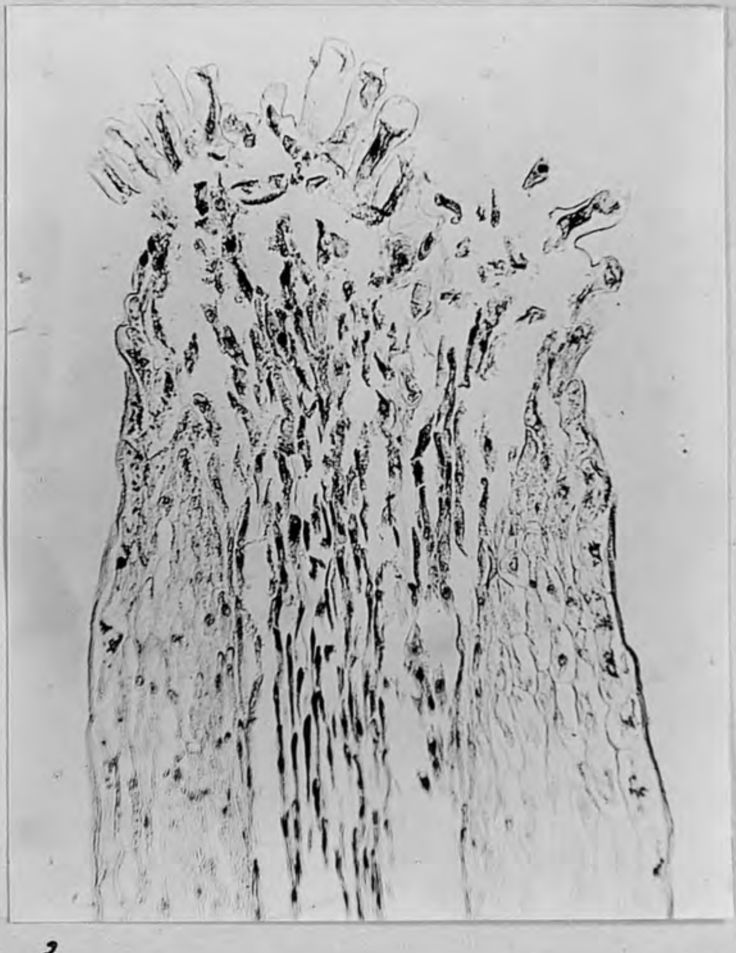

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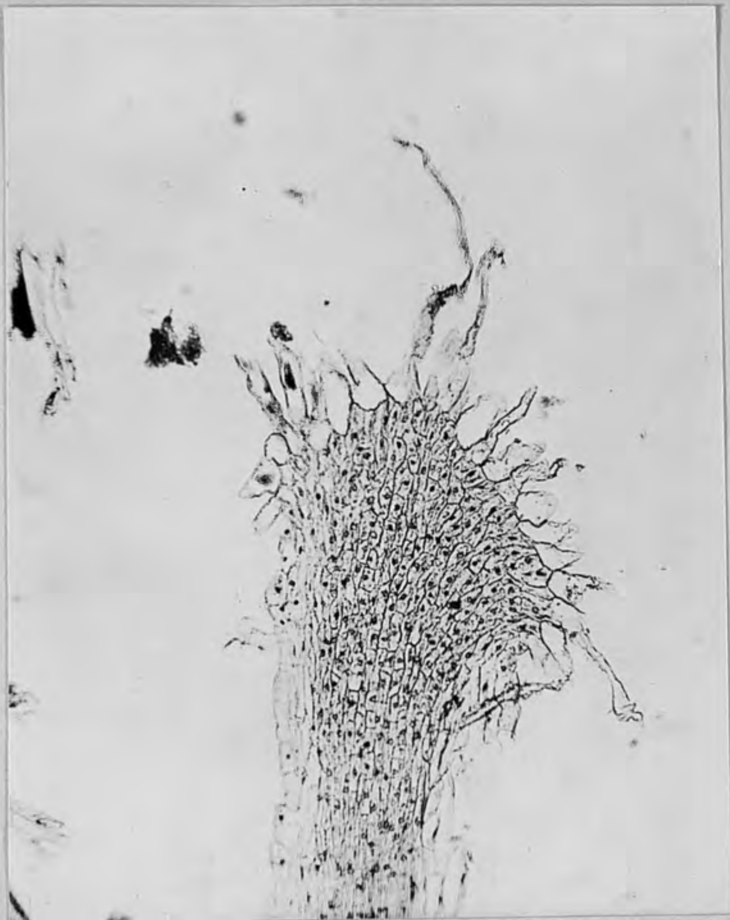

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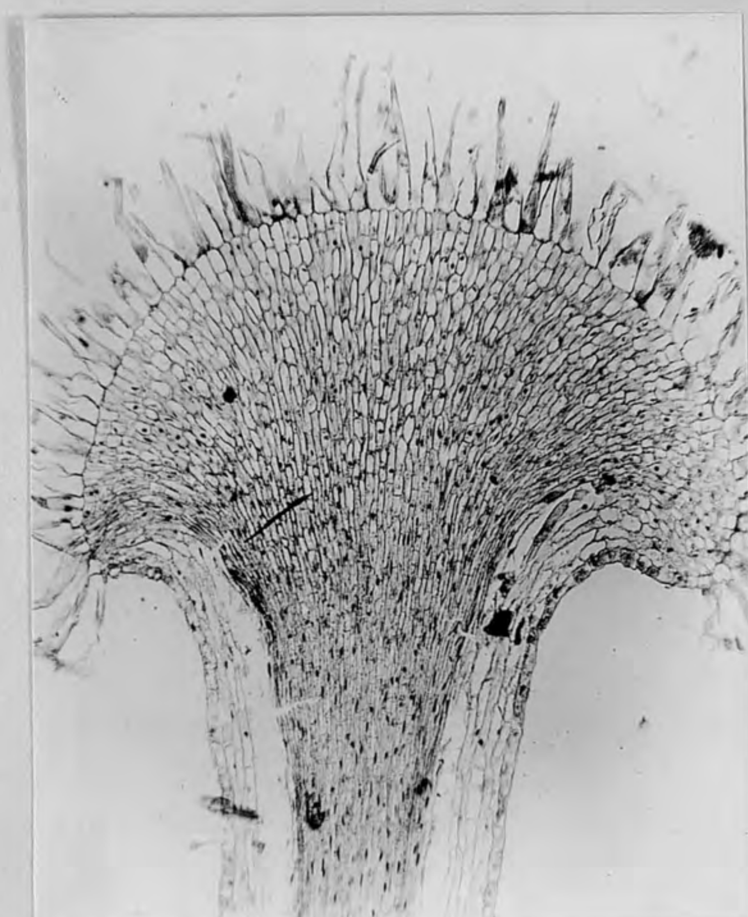

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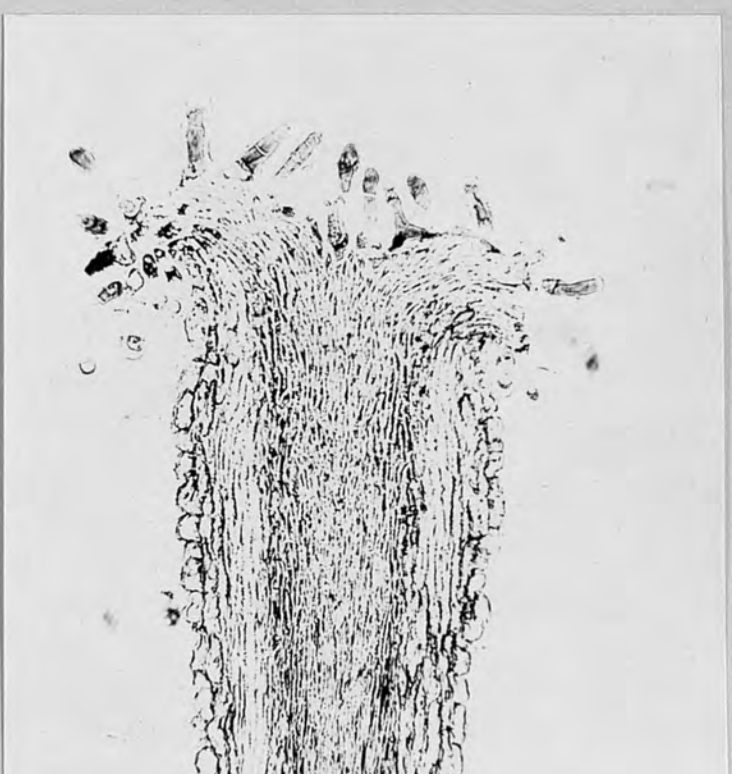

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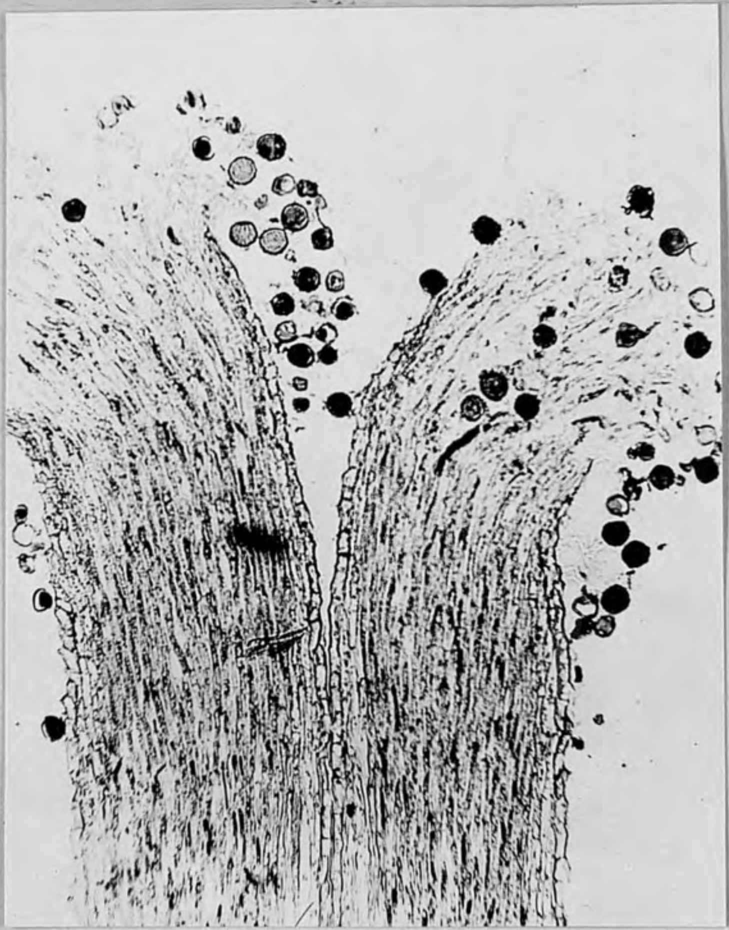

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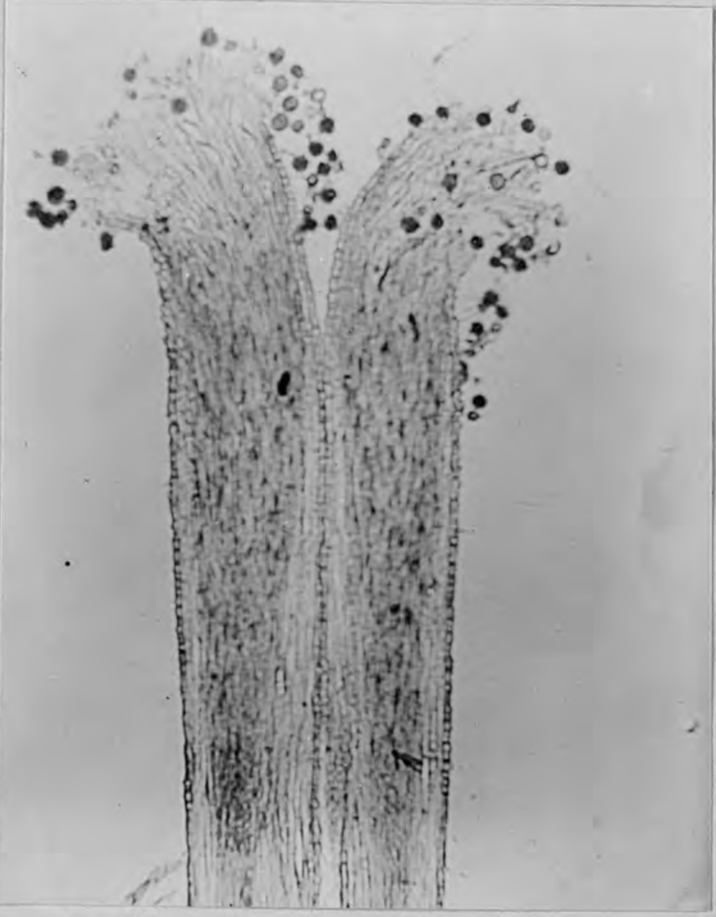

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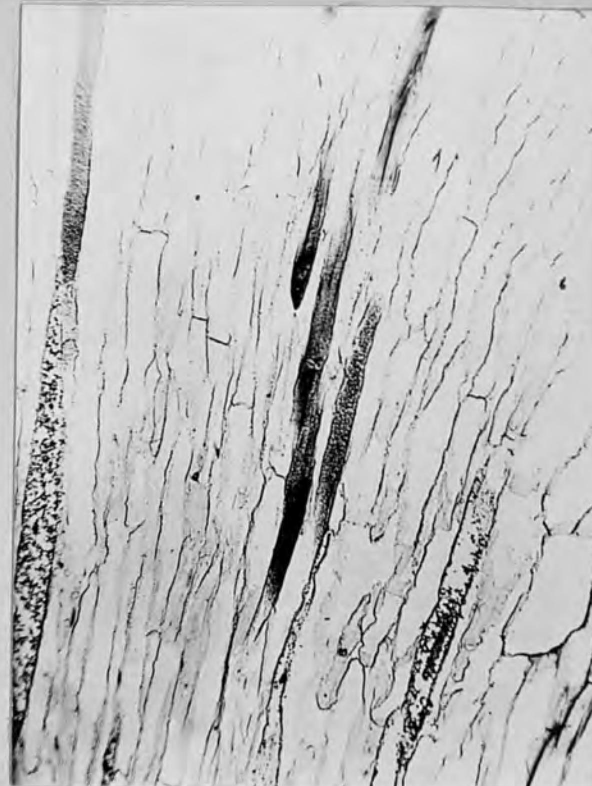

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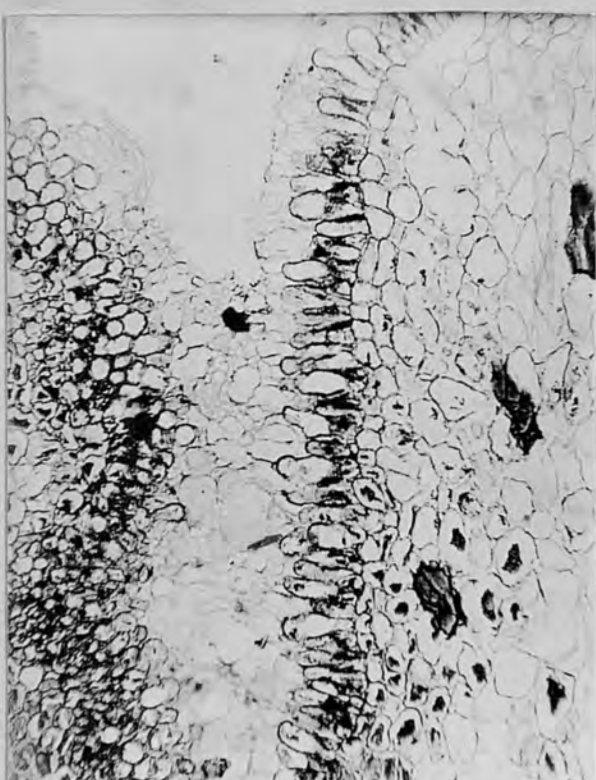


3.

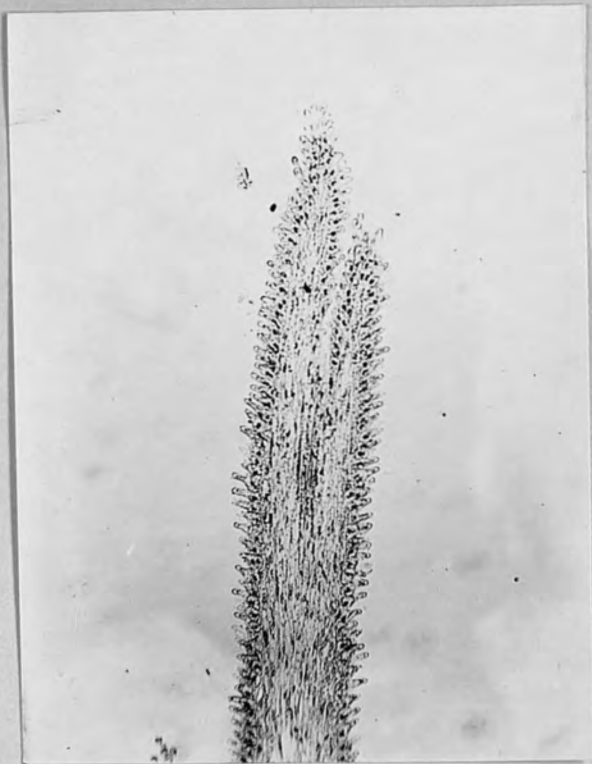
4.

Plate XXIII.

1.

2.

Plate XXIV



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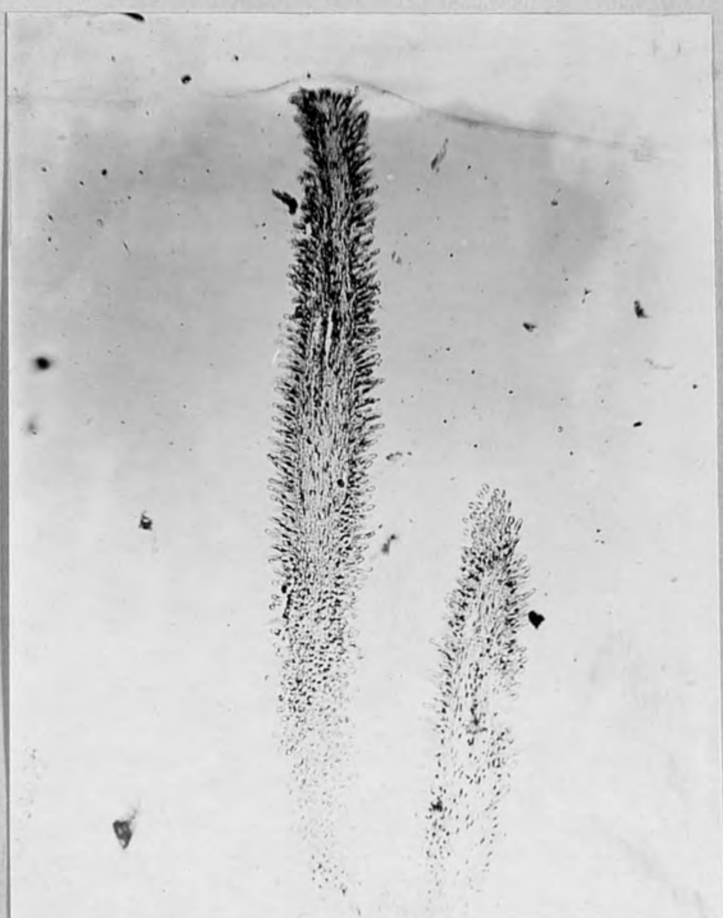

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Plate XXV.

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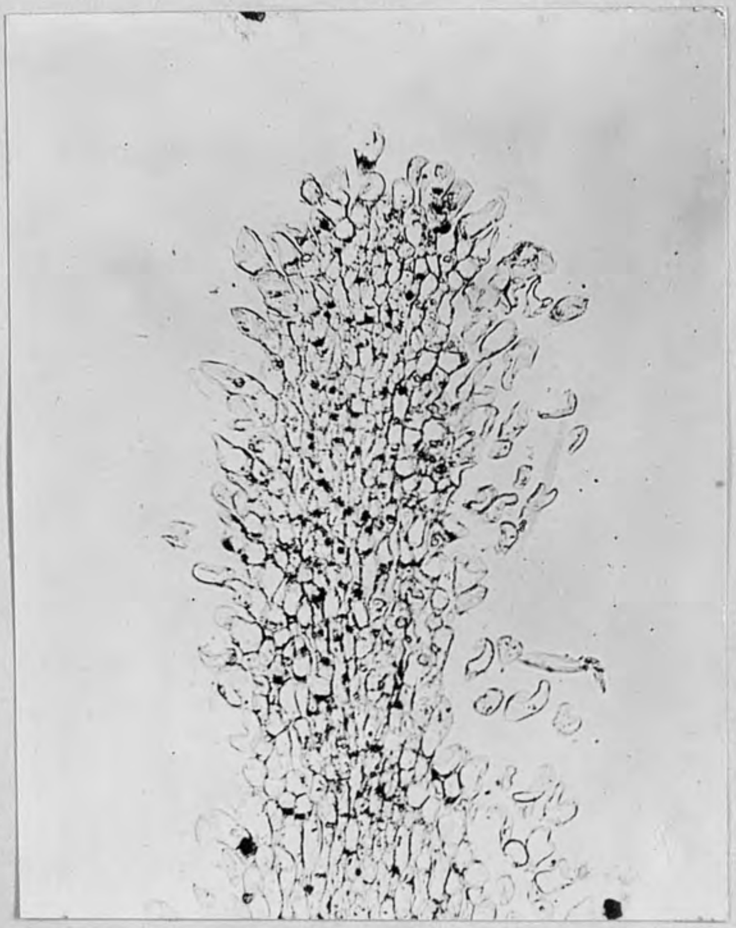

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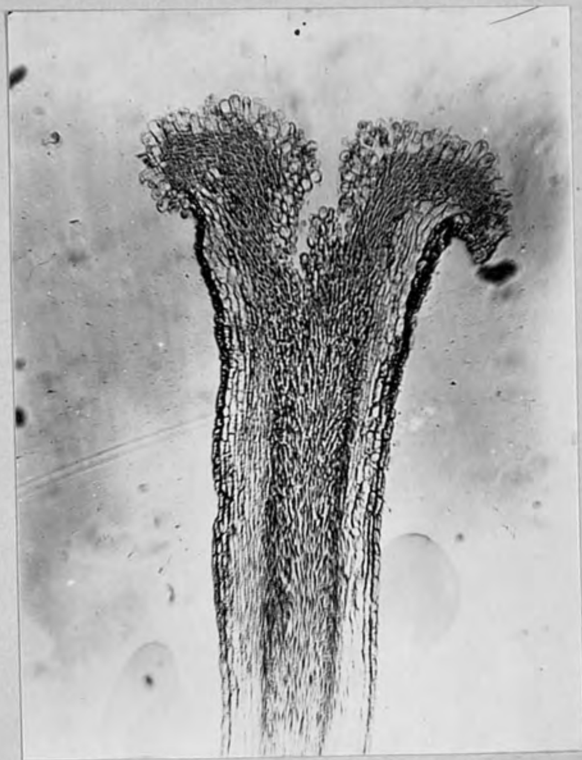
Plate XXVI



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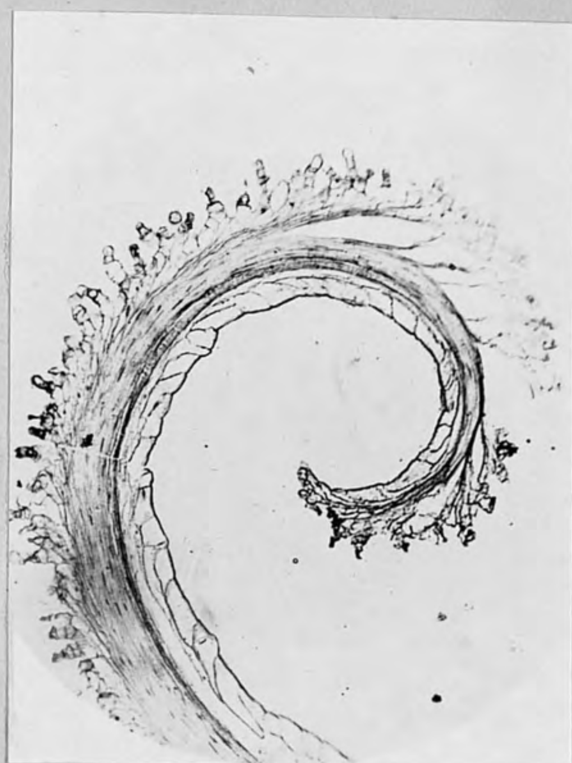

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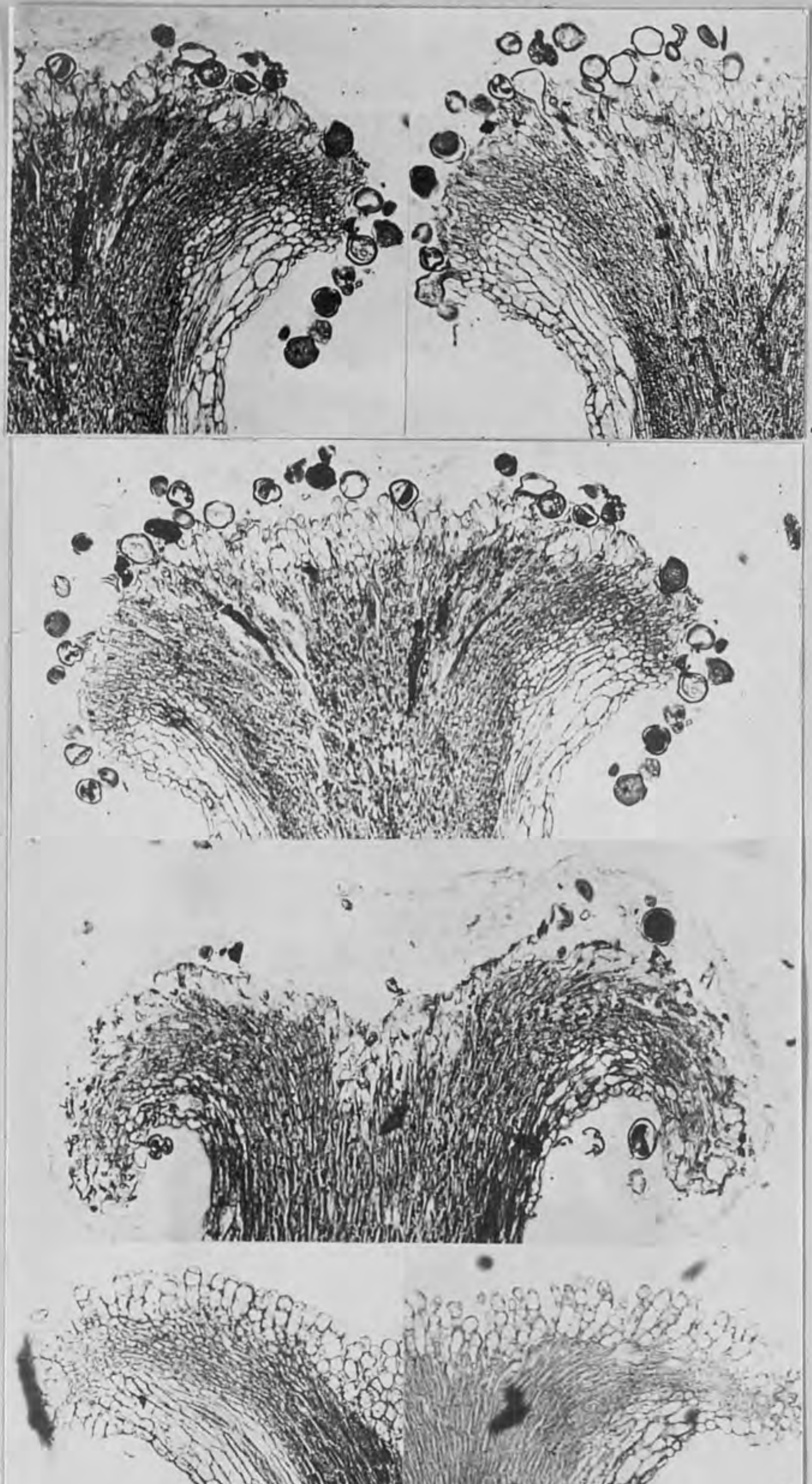
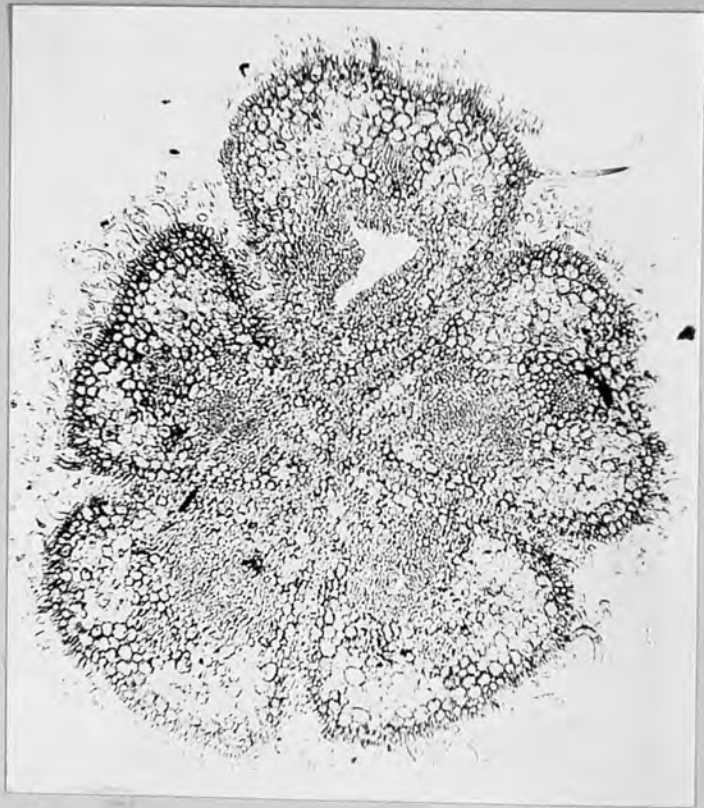
Plate XXVII



1. 2. 3. 4. 5. 6. 7. 8.

Plate XXVIII



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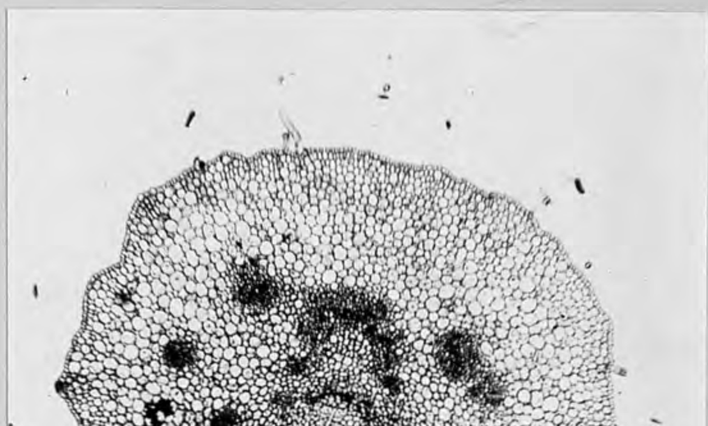
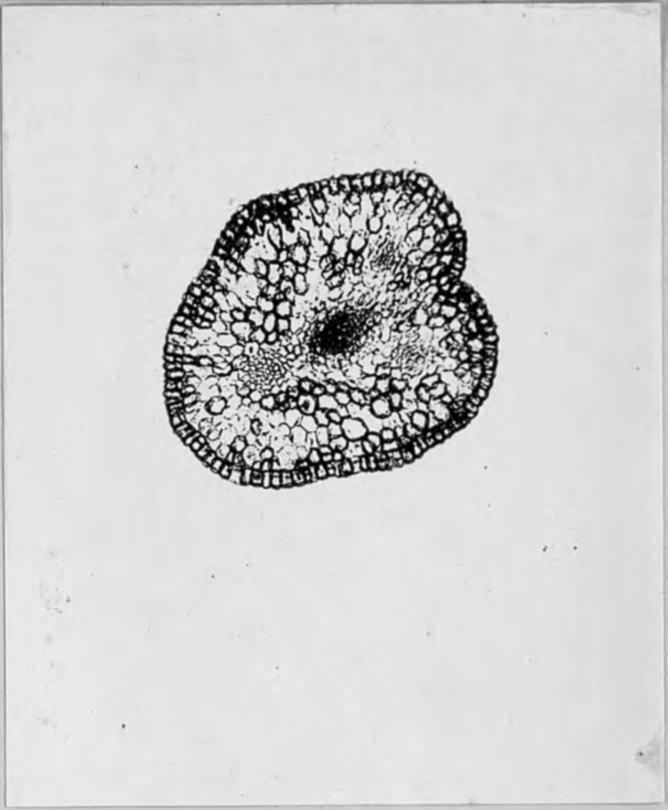


2.

Plate XXIX.



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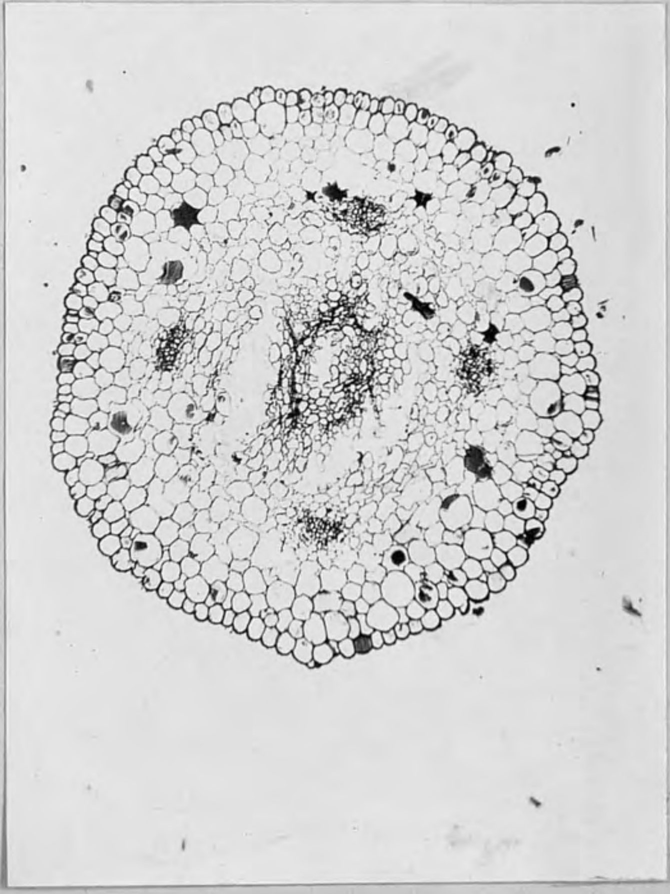

2.