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Abortive Stamens and Carpels in Lychnis Alba

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ABORTIVE STAMENS AND CARPELS IN LYCHNIS ALBA

BY

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Introduction

Botanists have long been interested in the manner of "sex expression" in plants. Some plants have perfect flowers, that is, both sexes are expressed in a single flower, with no variation from this condition within the species. Other plants have flowers in which only one or the other sex is expressed, while still other plants occur in which "pure staminate or pure carpellate and all intermediate degrees" occur. Schaffner (7)

Schaffner (7) was able to control sex expression in Arisaema triphyllum, (Indian Turnip or Jack in the Pulpit) to such an extent as to even reverse it by changing the environment. His conclusion was, in part, that "sex in Arisaema is dependent on a functional state and not on hereditary factors; that the sexual state is readily controllable and is reversible in either direction, male to female, female to male and back again; and that the dimorphism which appears in the inflorescences of these diploid organisms can not be due to homozygous and heterozygous factors or chromosome constitution."

A plant of Salix petiolaris was found by Chamberlain (1) that exhibited a "most surprising variety of sports." Pistils were found with microsporangia borne on stalks that closely resembled the placenta on which the ovules were borne. Both staminate and pistillate catkins were found on this plant and also others in which two stamens and one pistil, or one stamen and one pistil came from the same bract.

Simons (9) working with Cucumis sativus came to the conclusion that "each flower of Cucumis is essentially perfect, but due to environmental factors suppressing the potentialities of the chromosomes for the expression of certain organs, abortion of stamens or pistils <u>usually</u> occur."

Shull (8) quotes Strasburger on hermaphroditism in Lychnis diocea as being due to infection by Ustilago violacea. This was confirmed by Doncaster quoted by Shull (8) by artificial infection of Lychnis with Ustilago.

Correns (2) was successful in shifting the sex ratio in Lychnis by varying the amount of pollen applied, his conclusion was that "unequal production of heterogametes and unequal growth of pollen tubes modifies the sex ratio in Lychnis."

Studying chromosome behavior in Elodea, Santos (6) arrives at a conclusion somewhat different from the others mentioned that "segregation of sex factors takes place during sporogenesis." Santos (6) quotes Marchols as being "unable to alter sexes in Mosses by changing environmental conditions."

In view of the literature cited above, and the conclusions reached by the several investigators this present investigation was undertaken with the hope of contributing something in the search for an explanatory mechanism of sex determination in seed plants. The effort being first, to see whether or not flowers that are apparently unisexual are fundamentally so.

Material and Methods

Pistillate and staminate flowers of Lychnis alba, (Figs. 1, 2 and 3) known also as White cockle or White Campion, were gathered from June 17 to July 11, 1929, most of them from the Agronomy Station Farm, Brookings, South Dakota.

The material was collected from plants wherever they happened to be growing on the lawn. Moisture conditions were about the same, with one possible exception, some plants were located rather well under a porch. The lawn was made on a place, filled in with clay, old plaster, concrete, etc, and with a 6 or 8 inch black soil cover. Even then in places where the old plaster did show through there may have been very little difference in the available moisture or soluble minerals. If external environment has any influence on sex expression in Lychnis alba the environmental difference here was evidently not great enough to have a noticeable effect.

The material to be sectioned in paraffin was collected in all stages of development, buds singly and in clusters, and placed immediately in Formalin-acetic alcohol and imbedded in paraffin. Sections were cut 8 to 10 microns thick with a Spencer rotary microtome. Some of these sections, after being mounted on slides, were stained in Haidenhain's iron-alum haematoxylin, alone or with Orange G counter-stain. With few variations Yamanouchi's schedule for staining with Haidenhain's iron-alum haematoxylin was followed. The other sections were stained in Safranin, (in 50% alcohol) 8 to 12 hours, then placed in distilled water 10 to 12 hours, run up to 95% alcohol, counterstained with Light Green, cleared in xylol, then covered in Canadian Balsam. Some whole plants were collected, pressed, and kept for future reference as were also some specimens preserved in a 6% Formalin solution.

Succession of Floral Organs

The order of succession of the flower parts is acropetal i.e. sepals, petals, 2 whorls of stamens, and abortive pistil, or pistillodium, in staminate flowers; in pistillate flowers, the succession is sepals, petals, abortive stamens, or staminodia, and carpels. The stamens do not always develop in the same order in separate flowers nor even on opposite sides of the pistillodium in the same flower. In Fig. 20 it is evident that the outer cycle has developed, perhaps at the expense of the inner cycle, and both sides of the flower are the same.

A section of another staminate flower, older than the one in Fig. 20 shows approximately the same relative difference in stamen development on one side while on the other side it appears as if the two stamen primordia had grown up together. The staminate flowers as well as the pistillate flowers are consistant only in their variability. The youngest flowers observed had transverse diameters almost as great as their longitudinal diameters, and except for the terminal one, were subtended by a bract or leaf. The terminal flower had neither bract nor leaf between it and the next lower flower. In the very early developmental stage there are no visible differences between the staminate and the pistillate flowers, but differences soon develop. (Figs. 17, 18, 19).

Pistillate Flowers

The number of styles varies from 3 to 8 in pistillate flowers with 5 occurring more frequently than any other number in the specimens examined. The cross sections of styles of a young pistillate flower show that they are rounded at and near their tips, but successive sections progressively nearer the developing capsule are more or less heart shaped with the indentation towards the center and of course larger as the capsule is approached. (Fig. 5). The styles seem to approach one another and their edges fuse, or else we reach the level at which the tissue between the adjacent styles developed at the same rate and time as the styles themselves, thereby causing a continuous cylinder, the outer wall of which will later be the capsule wall. (Fig. 6).

The infolded margins of the carpels seem to fuse, where their dorsal surfaces are contiguous, or else they grow up simultaneously, and extend towards the center of the capsule. (Fig. 7). As these infolded margins approach one another in the center of the capsule (Fig. 8) they become more and more heart shaped but with extended, rather thick attachments instead of indentations (Fig. 9) and at a slightly lower level all centripetal ends of the fused margins of the carpels will constitute the axial placenta and completely fill the center of the capsule. (Fig. 10). The placenta is now a solid mass of tissue and the indentations of the heart shaped styles, or carpels, have developed into chambers or cavities of the capsule. (Fig. 11). Parts of the fused margins of the carpels form partition walls between the capsule cavities, and those portions of the carpels corresponding to the midrib region of leaves constitute the capsule wall. Before maturity these partitions break down, (Figs. 12 and 13) as in the Primrose, "thus leaving a mass of tissue in the basal portion of the ovary, the remnant of the axis, upon which the ovules are attached." Pool (5).

In Lychnis alba the mass of tissue that "fills the basal portion of the ovary" as in the Primrose, extends the full length of the ovary and there is an attachment between the top of the placenta and the inner surface of the tip of the capsule. (Fig. 14). The partitions that extend longitudinally between the cavities of the capsule break down before the attachment from the top of the"dome" to the capsule tip breaks. The capsule wall continues to expand after the placenta and ovules have ceased to increase in size so that in the mature capsule the cavity which is now single and continuous is not completely filled. At maturity there are no indications of partition walls nor of the attachment of the placenta to the inner surface of the capsule tip, though in young pistillate flowers these attachments are readily observed. From observations made in this investigation it may be concluded that in Lychnis alba the placenta is not the remnant of the axis but is composed of the fused margins of the carpels. The ovules are therefore of foliar origin, rather than cauline.

Staminodia of various sizes and shapes occurred in every pistillate flower examined. Fig. 15, a longitudinal section, is fairly representative of those observed. One staminodium was found that in cross section showed a well defined cavity (Fig. 16). There was no definite layer of cells lining the cavity that could be compared with the wall of the anther chamber or the tapetal layer but it was equally evident that the cavity was not due to cell disintegration.

The pistillate flower of Lychnis alba seems to be potentially bisexual as indicated by the aborted stamens that occur in the same position in pistillate flowers as the normal stamens occur in staminate flowers.

Staminate Flowers

As mentioned before there is no noticeable difference in very young pistillate and very young staminate buds, (Figs. 17, 18 and 19) but a difference is soon noticed in that part of the receptacle that develops into the ovary in the pistillate flower and into the pistillodium in the staminate flowers.

Gray (4) describes Lychnis as having "5 styles, rarely 4, and pod opening with twice as many teeth; otherwise as Silene," Lychnis alba, if like Silene, would then have 10 stamens and flowers dioecious or polygamous; but only 2 flowers were found with 10 mature stamens, and no polygamous plants were found. In cross sections of almost matured pistillate flowers ten staminodia were found in two cycles, with alternate ones, those opposite the petals, taller or further developed than those alternate with the petals.

Of six mature staminate flowers disected, two were found to have 10 mature stamens, one had 7 mature stamens, one 8, one 9, and in the other no two stamens were of the same size. Two very small stamens were found in one flower that were barely visible to the eye and they remained attached to the petal when it was removed, while in the other flowers, except where 10 mature stamens occurred, it seemed usual for one of the larger stamens to be attached to each petal and the smaller ones alternate with the petals. The stamens are borne on the stipe or stalk of the ovary and in the pistillate plant 10 staminodia occur, usually the staminodia opposite the petals appear before the ones opposite the sepals. There are potentially 2 cycles of stamens in these flowers with a tendency for the outer cycle to abort even in staminate flowers. (Fig. 19).

The apex of the floral axis in the staminate flower still seems inclined toward production of some kind of carpel representative. No specimen of staminate flower was found in which separate carpels could be distinguished, and none in which any lateral growth had fused to form anything like an ovary cavity. But at the point, where in pistillate flowers carpels occur, a short, solid, cylindrical mass of tissue develops resembling a style in appearance, though not in origin; this is the pistillodium. In the receptacle just below the pistillodium, a rather large group of cells was seen to be disorganizing (Figs. 21 and 22) but the cavity resulting from this disorganization could scarcely be called or even compared with a true ovary cavity because there is no regular layer of cells lining the cavity ; the cavity is not in the right position; and moreover, an ovary cavity is not caused by the disorganization of any cells in a solid receptacle.

It is believed that the staminate flowers of Lychnis alba are also potentially bisexual as indicated by the pistillodium that was found to occur in every staminate flower examined.

Abnormalities

Some pistillate buds were found that showed interesting digressions from the manner of development described for pistillate flowers. In one bud ovules were developing at irregular places before the margins of the carpels had fused to form a solid placenta, and, as in other cases of irregular ovule development, this was accompanied by the appearance of small groups of very peculiar cells, that in this instance occurred in the capsule wall (Fig. 23). A section of the same capsule showing that the group of cells, mentioned above, were torn loose from the adjoining cells by the microtome knife as if there was a difference in consistency of wall between these cells and normal ones is pictured in Fig. 24.

Another capsule was found, where on a single section three ovules could be seen attached to three different surfaces within the capsule. One was attached to the inner surface of the capsule wall, one to a partition wall and a third in a cavity within the placenta with its funiculus pointing toward the capsule wall (Fig. 25). The ovule attached to the capsule wall is shown under higher magnification in Fig. 26.

Abnormal conditions are shown in Fig. 27 in that the region occupied normally by the placenta is an empty space. The margins of the carpels that normally develop into the placenta which in turn bears the ovules, are here shown developing ovules directly. While ovules may be borne on any "free surface within the ovary", Coulter and Chamberlain (3), it is usual, except among primitive families, for the ovules in a given species to be borne on the same surface.

The groups of abortive, or peculiar, cells that usually occurred along with other irregularities in the pistillate flowers were first noticed in the region of a staminodium. (Figs. 28 and 29). It was found later that groups of these cells occurred in various parts of the pistillate flowers: in the capsule wall (Fig. 23), in the staminodia or receptacle (Fig. 28), in the placenta (Figs. 30 and 31) and on the border line between the capsule wall and petal (Fig. 32). One instance was found where one group of these cells occurred on the border line of the petal and capsule and in the same petal, toward its margin a group of these cells occurred entirely free from the capsule of the flower (Fig. 33). Another section was found of a petal in which a group of these cells occurred, so large as to reach almost across the thickness of the petal near its midrib, (Fig. 34). The group of cells shown in Fig. 35 is the same as shown in Fig. 31 but under greater magnification. Another group of these cells highly magnified is shown in Fig. 36.

In every instance that groups of these cells occurred, regardless of their position in the flower, they sectioned with greater difficulty than did the neighboring cells and were always torn loose from them on one side at least. Because of the location and appearance of the groups of these peculiar cells that were first noticed it was thought that they might be abortive microspore mother cells. Chamberlain (1) reports a "strange sport" of Salix petiolaris "sometimes the microsporangia were borne upon long stalks, some times on placenta-like outgrowths of the carpel, and sometimes imbedded in the carpel wall." He describes another flower of this same "strange sport" as having "two quadrilocular stamens with filaments united below and with the connective prolonged above into a stigma." Writing of microsporangia borne inside the ovaries he says "the microspore development was sometimes normal but as often feeble and abortive."

Sawyer (10) found ovules borne on stamens of Podophyllum peltatum "usually on the lower part of the expanded portion, not far from the pollen sacs."

The idea that these groups of peculiar cells were microspore mother cells was abandoned upon closer study for several reasons: there was no tapetal layer surrounding them; the denser color seemed to be due to the cell walls rather than the cell contents; these cells and their nuclei were not much different in size and shape from the normal cells; and the normal microspore mother cells, found in the anthers, (Fig. 21) sectioned as if they were of the same consistency as the neighboring cells.

The question remains whether there is a difference in the chromatic content of the usual egg and sperm in plants. If such a difference normally occurs, at what point does the differentiation take place? Chamberlain (1) found stamens borne within the ovary of Salix petiolaris and Sawyer (10) found ovules borne on stamens so in these cases the differentiation into microspore and megaspore must have taken place later than the differentiation into stamens and carpels.

While purely speculative, the conditions just described for Salix petiolaris may occur also in Lychnis alba if the abnormal cell groups be considered as pollen mother cells.

Discussion

Because of the presence of both stamen and carpel representatives in each flower of Lychnis alba this species is believed to be potentially bisexual. Due probably to some undetermined factor or group of factors, only one or the other set of organs develops while the remaining set aborts. Simons (9) writes that "each flower of Cucumis is essentially perfect, but due to environmental factors suppressing the potentialities of the chromosomes for expression of certain organs, abortion of stamens or pistils usually occurs." It seems that sex is determined rather early in the life history of these plants, possibly, as suggested by Correns (2) as early as fertilization. When a plant is either wholly staminate or wholly pistillate, sex determination must have occurred at the origin of the plant i.e. at the time of fertilization or very soon after. If branches of the same plant are unlike in sex, the determining factor must be physiological, and active in the stem before the branches are put out. If sex determination is delayed until the flowers on the ultimate branches are produced, the differentiation would then occur in the receptacle of the flower and a "border-line" case could easily be imagined in which a microsporangium and an ovule might develop side by side within a pistil, as reported by Chamberlain (1) for Salix petiolaris.

Summary

The flowers of Lychnis alba are believed to be potentially bisexual.

An unknown factor or group of factors causes one or the other set of sex organs to fail to develop.

The placenta in Lychnis alba is at the end of the axis but is not itself any part of the axis.

The placenta is carpellary and therefore the ovules are foliar in origin.

There are two cycles of five stamens each and when less than ten stamens mature in the staminate flower the inner cycle is usually the one that tends to abort though there is some variation.

Abnormal ovule and placenta development was accompanied by the occurrence of groups of abnormal cells that seemed to occur most frequently in or near the staminodia but also occurred in different parts of the capsule, placenta and also in petals. As to the interpretation of these cells, we are still in the dark.

It is a pleasure at this time to acknowledge the stimulating suggestions and criticisms that were freely given by Dr. Ward L. Miller, of the South Dakota State College of Agriculture and Mechanic Arts, during the preparation of this paper.

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EXPLANATION OF PLATES

PLATE I

Fig. 1 Staminate and pistillate plants of Lychnis alba.

Fig. 2 Pistillate plants of Lychnis alba.

Fig. 3 Staminate flowers of Lychnis alba.

Fig. 4 Longitudinal section of pistillate bud: b,bract; bu,very young bud; c, capsule; p,petal; s,stamen; se,sepal. X77

PLATE II

Fig. 5 Cross section of pistillate bud: i,indentation in style; s,cross section of style near its tip; se,sepal. X77

Fig. 6 Cross section of pistillate bud near base of styles: ad,point where margins of carpels adhere to each other or fuse; i,indentation of style. X77

Fig. 7 Cross section of pistillate bud near upper end of capsule: ad,point where margins of carpels have fused; i,indentation of styles; se,sepal. X77

Fig. 8 Cross section of pistillate bud: ad,points formed by fused margins of carpels; i,indentation of style becoming capsule cavity; se,sepal. X77

PLATE III

Fig. 9 Cross section of pistillate bud: i,indentation of style becoming capsule cavity; p,points of fused margins of carpels that will form the placenta; w, portion of carpels that will become partition walls within capsule. X77

Fig. 10 Cross section of pistillate bud: i,capsule cavity; p,solid central placenta has just formed. X77

Fig. 11 Cross section of pistillate bud: ca,capsule wall; i,capsule cavity developed from indentation of styles; o,point where ovules will develop;

w,partition wall. X77

Fig. 12 Cross section of older flower: ca,capsule wall; o,ovule; pe,petal; w, partition wall breaking down. X77

PLATE IV

Fig. 13 Cross section of older capsule: ca,capsule wall; o,ovule; p,placenta; w,remnant of partition wall. X77

Fig. 14 Longitudinal section of young pistillate bud: a,attachment of placenta to capsule tip; ca,capsule wall; o,ovules; p,placenta; s,style; se, sepal. X77

Fig. 15 Longitudinal section of capsule: ca,capsule; st,staminodia. X77

Fig. 16 Cross section of staminodia: c,cavity in staminodia; ca,capsule wall; pe,petal; st,staminodia. X364

PLATE V

Fig. 17 Longitudinal section of staminate bud. X77

Fig. 18 Longitudinal section of pistillate bud: b,bract; c,carpellary region; l,leaf; pe,petal; se,sepal; st,stamen primordia. X77

Fig. 19 Longitudinal section of staminate bud: b,bract; c,carpellary region; pe,petal; se,sepal; st,stamen primordia. X77

Fig. 20 Longitudinal section of staminate bud: pe,petal; ps,pistillodium; se, sepal; sp,primordia of inner cycle of stamens; st,stamen in outer cycle of stamens. X77

PLATE VI

Fig. 21 Longitudinal section of staminate flower: d,cells disorganizing; m, microspores; ms,microspore mother cells; pe,petal; ps,pistillodium; se,sepal. X77

Fig. 22 From same section as Fig. 21: d,disorganizing cells at base of pistillodium; ps,pistillodium. X364

Fig. 23 Cross section of abnormal pistillate bud: o,irregular ovule development; pc,group of peculiar cells. X77

Fig. 24 Cross section of same bud as Fig. 23: o,irregular ovule development; p,irregular placenta; pc,place where group of peculiar cells were torn loose. X77

PLATE VII

Fig. 25 Cross section near base of capsule: a,ovule attached to capsule wall;
b, b,ovule within cavity in placenta; c,ovule attached to partition wall; x,abnormal placenta. X77

Fig. 26 Portion of same section as Fig. 25: a,ovule attached to capsule wall; ca,capsule wall; o,other abnormal ovules. X364

Fig. 27 Cross section of same capsule as Fig. 25: ca,capsule wall; o,abnormal ovules; x,cavity in the position normally occupied by continuous tissue of placenta. X364

Fig. 28 Longitudinal section of pistillate flower: ca,capsule wall; h,hole left by group of peculiar cells when torn out; o,normal ovules; p, normal placenta; pc,group of peculiar cells; pe,petal; se,sepal; st,staminodia. X77

PLATE VIII

Fig. 29 Tangential section of pistillate flower: p,placenta; pc,group of peculiar cells; st,staminodia. X77

Fig. 30 Tangential section of pistillate flower: p,placenta; pc,group of peculiar cells. X77

Fig. 31 Cross section of pistillate flower: p,placenta; pc,group of peculiar cells. X77

Fig. 32 Cross section of pistillate flower: ca,capsule wall; pc,group of peculiar cells on border line between capsule wall and petal; pe,petal. X77

PLATE IX

Fig. 33 Cross section of pistillate flower: ca,capsule wall; pc,group of peculiar cells; pe,petal. X77

Fig. 34 Cross section of pistillate flower: ca, capsule wall; pc,group of peculiar cells; pe,petal. X77

Fig. 35 From same section as Fig. 31: pc,group of peculiar cells; t,normal tissue. X364

Fig. 36 Group of peculiar cells: pc,group of peculiar cells; t,normal tissue. X364

PLATE I

Fig. 1

Fig. 2

Fig. 3

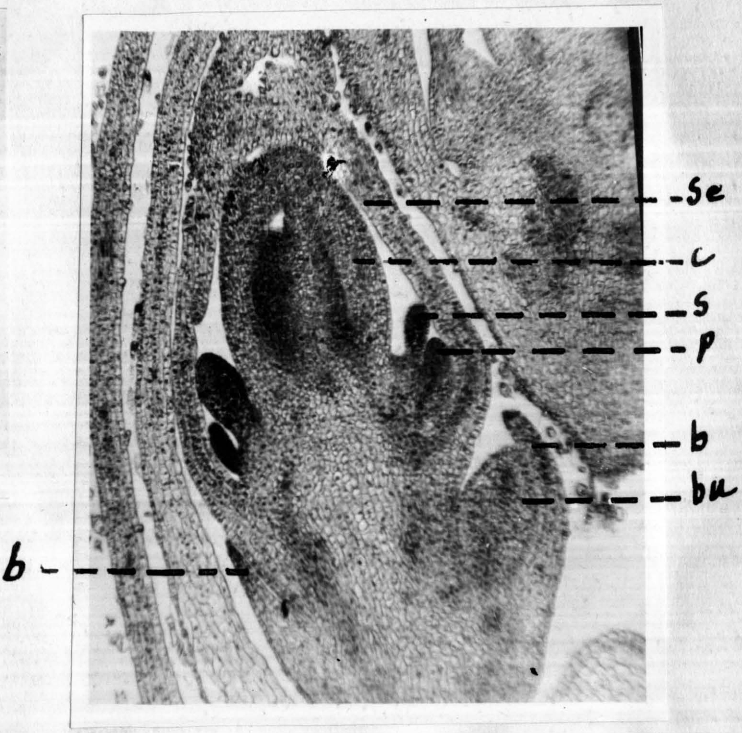
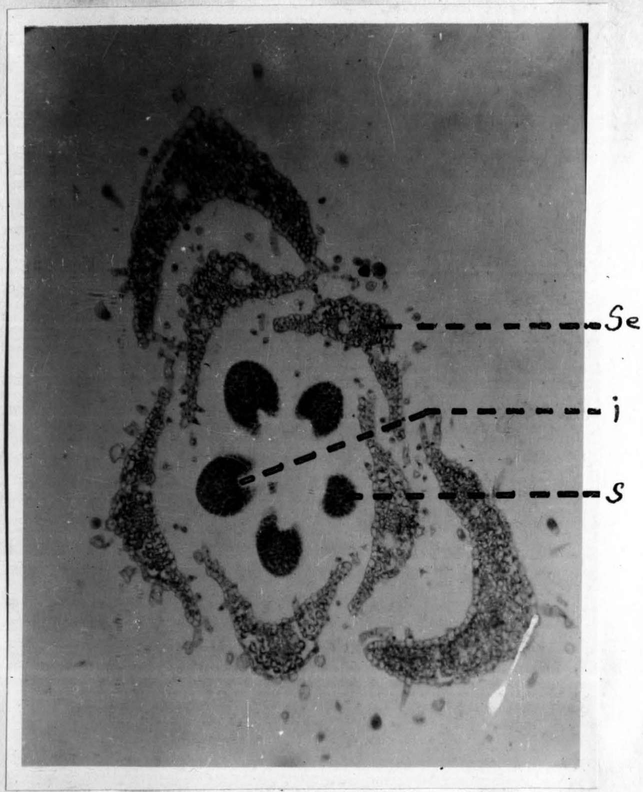


Fig. 4



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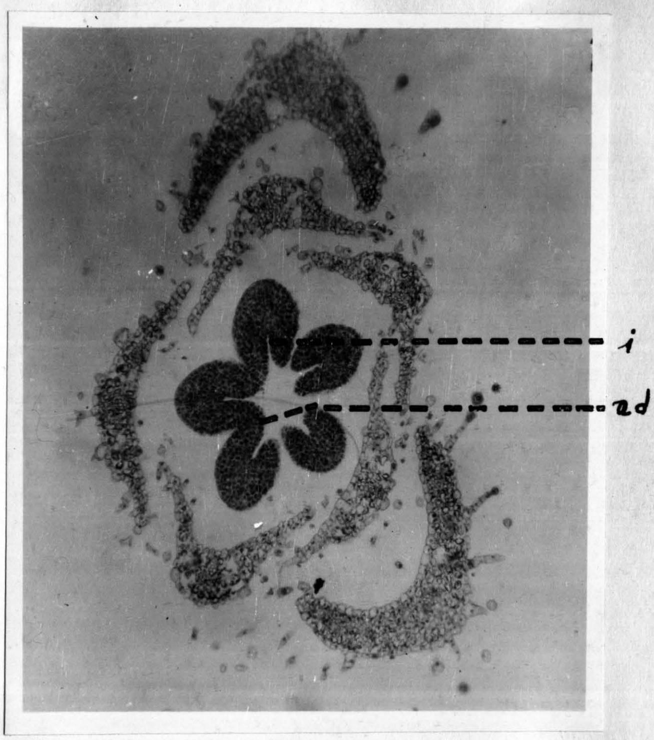

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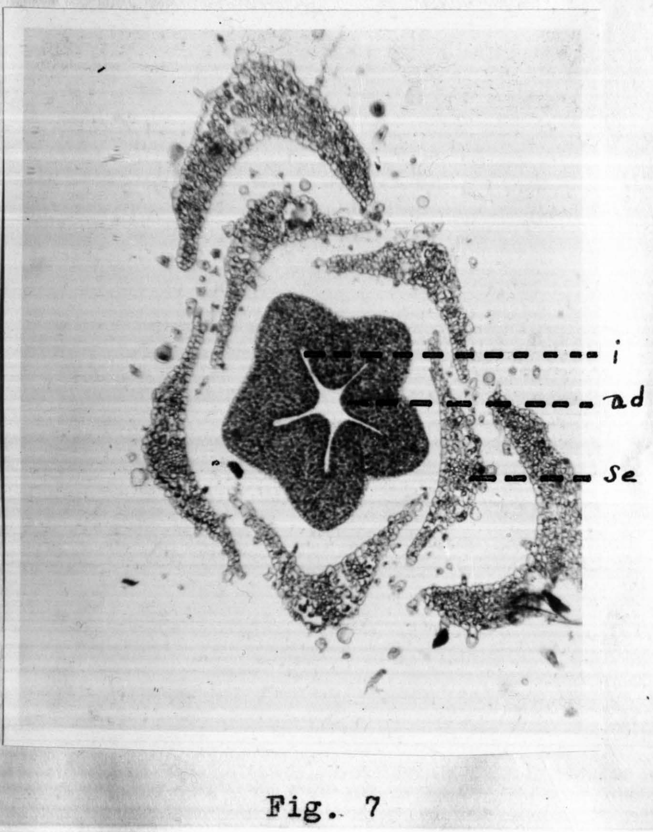

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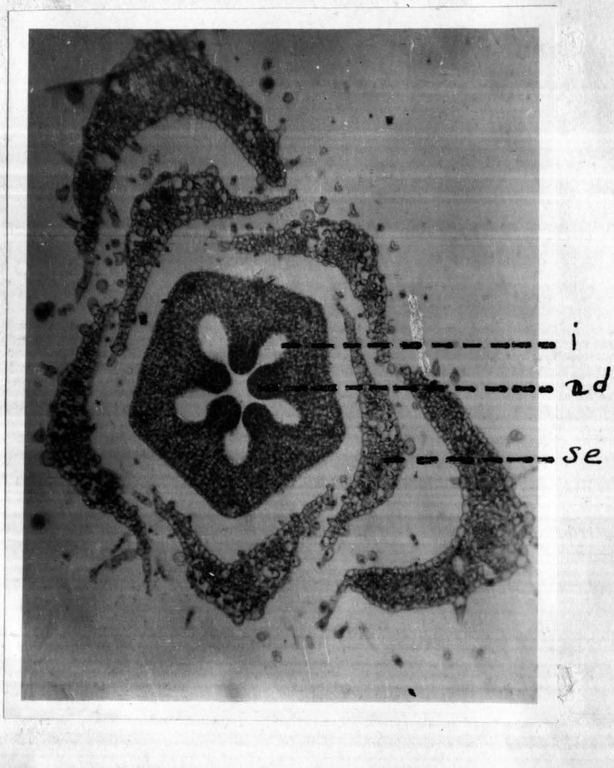

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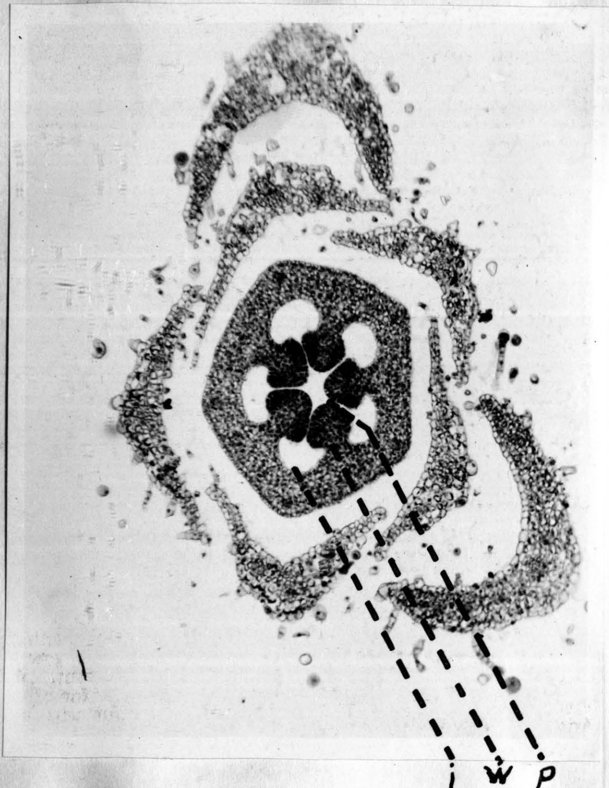
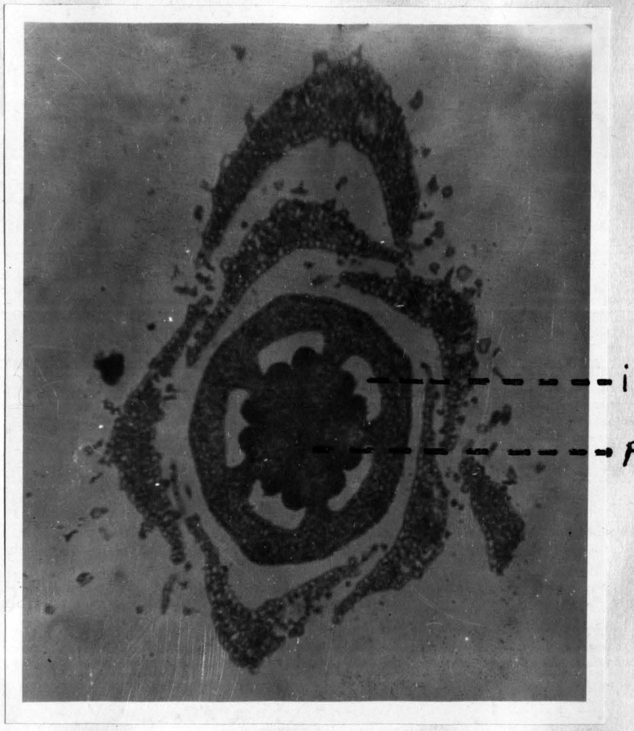


Fig. 9



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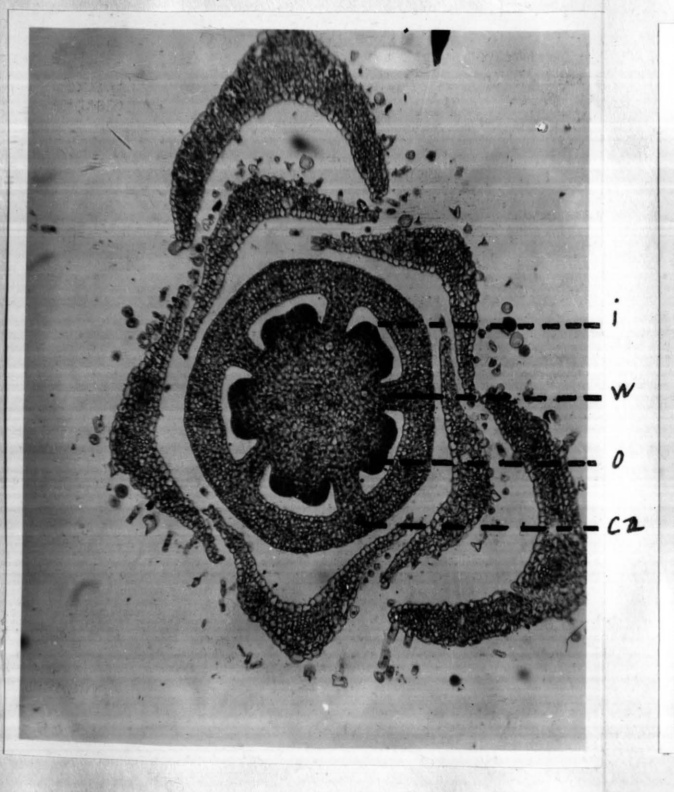

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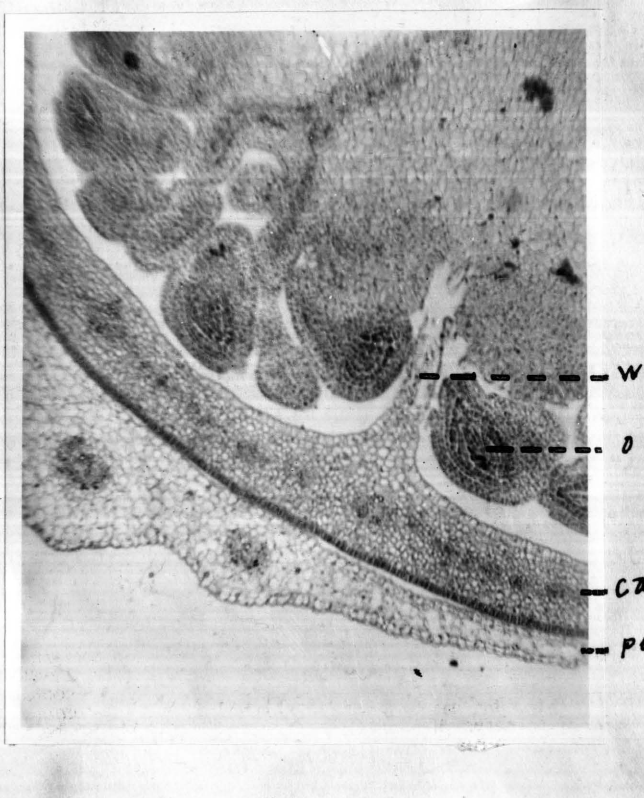

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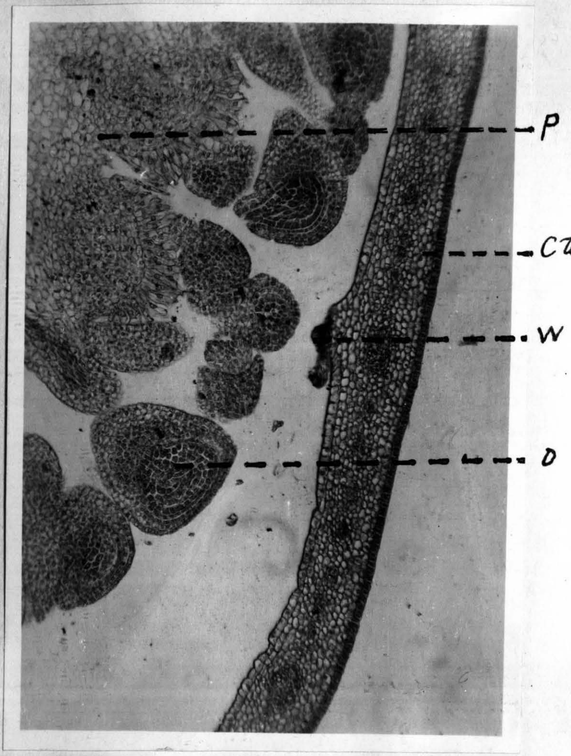
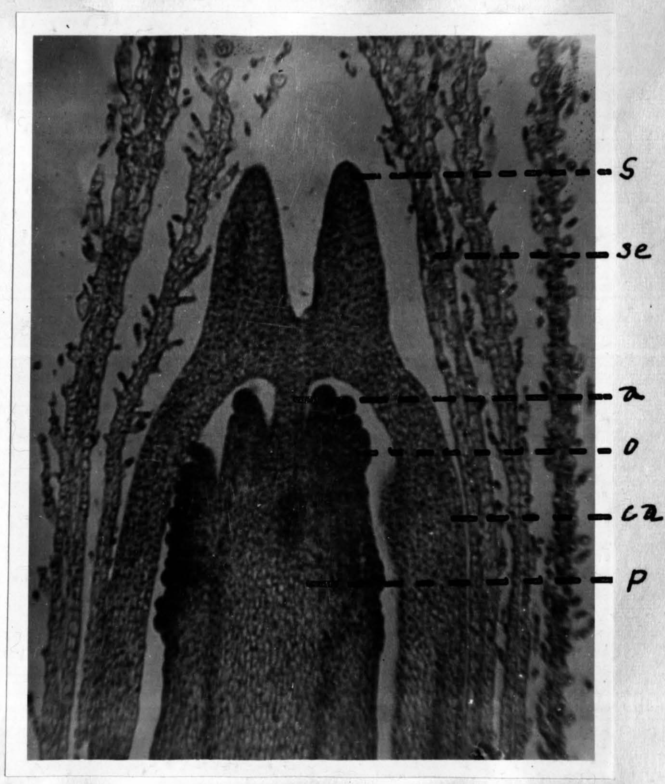


Fig. 13



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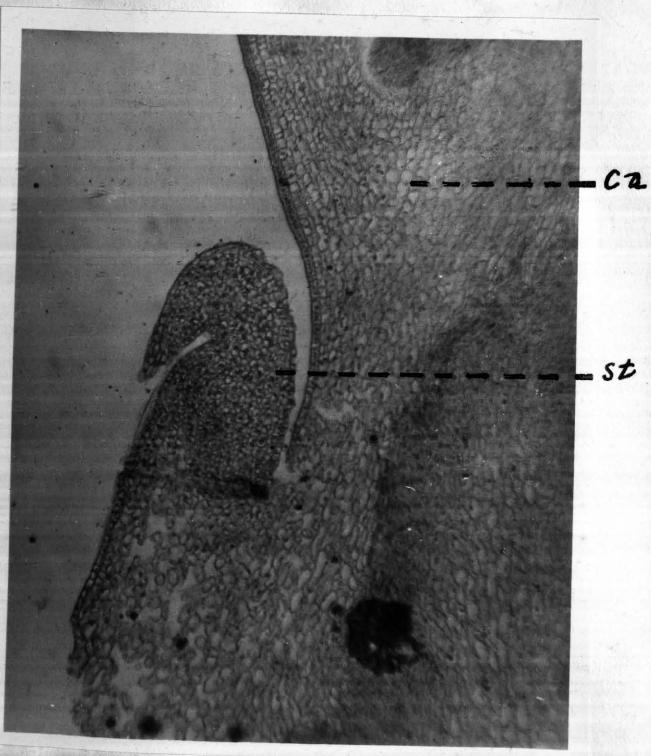


Fig. 15

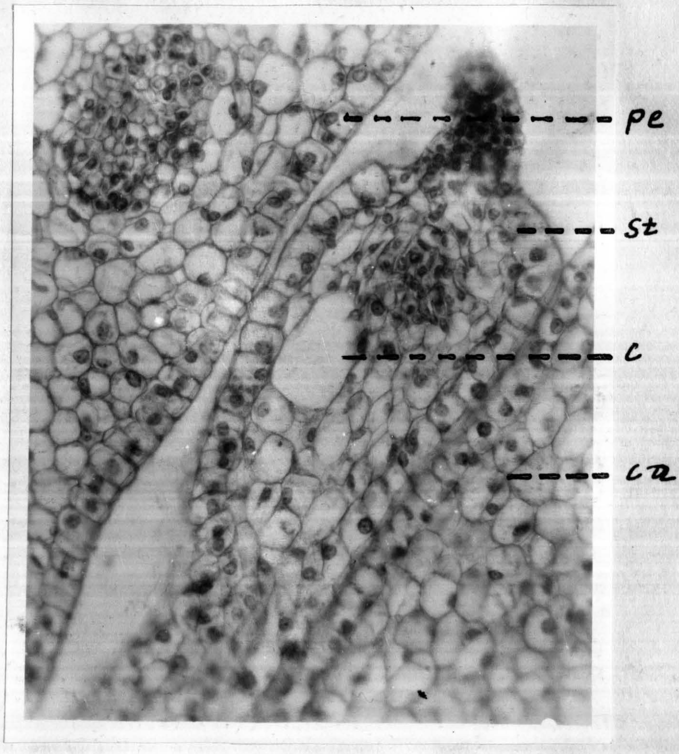
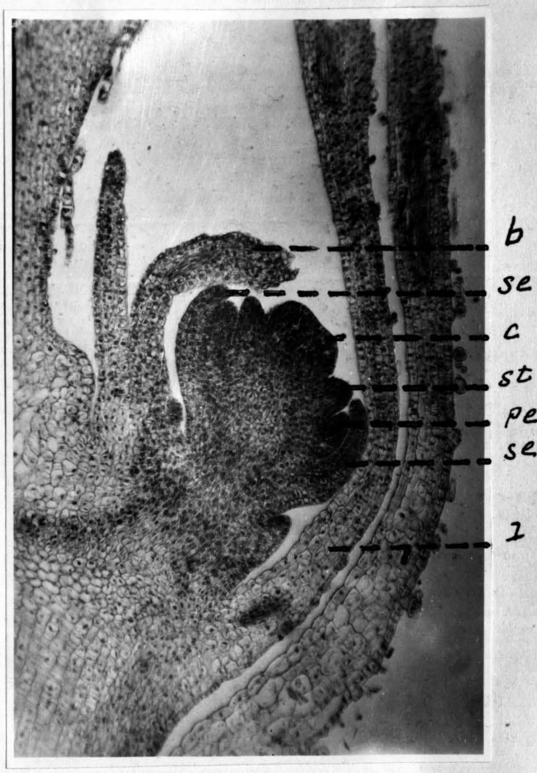


Fig. 16

Fig. 17



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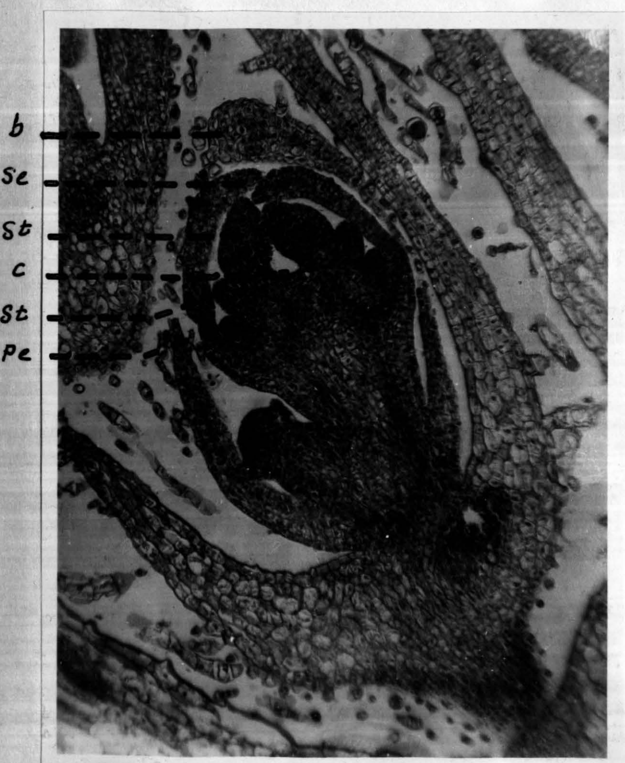

Fig. 19

Fig. 20

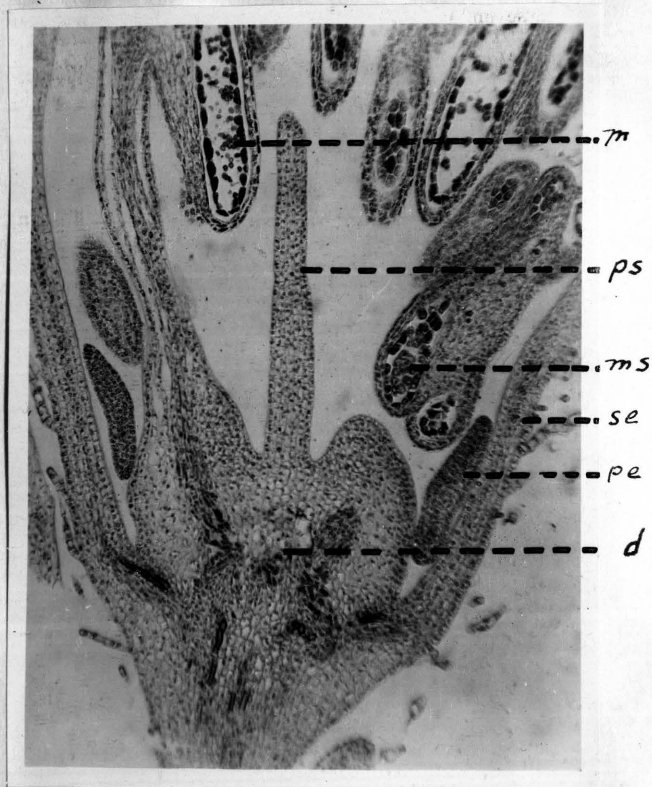

Fig. 21

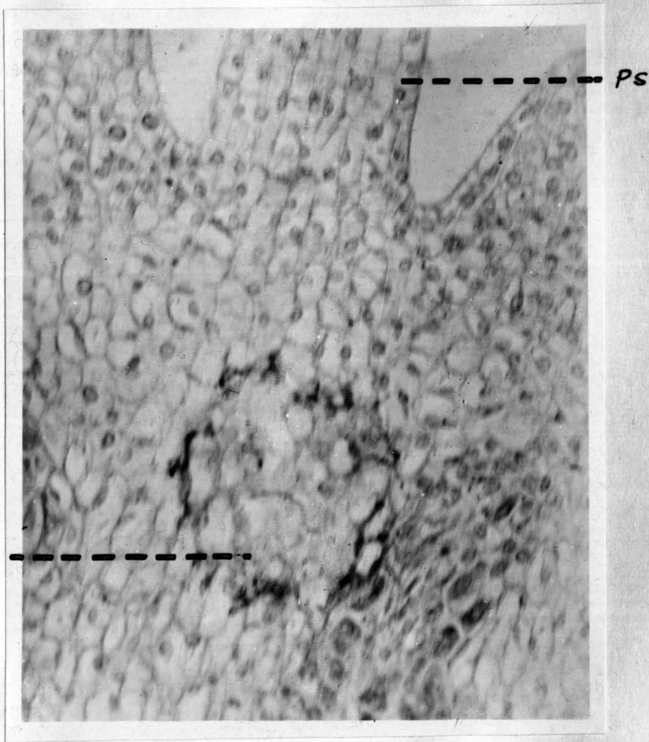

Fig. 22

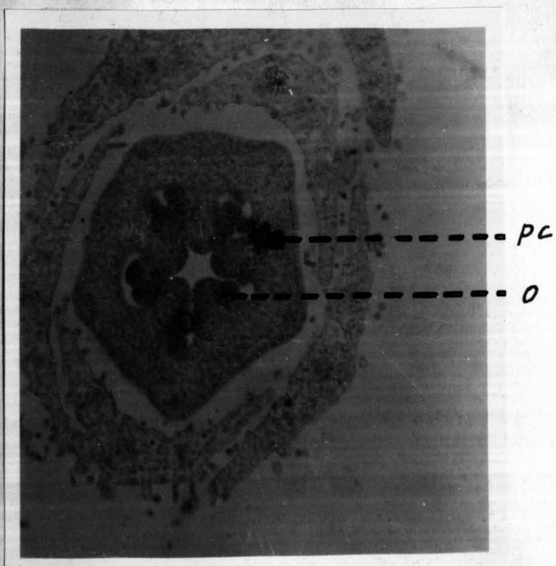

Fig. 23

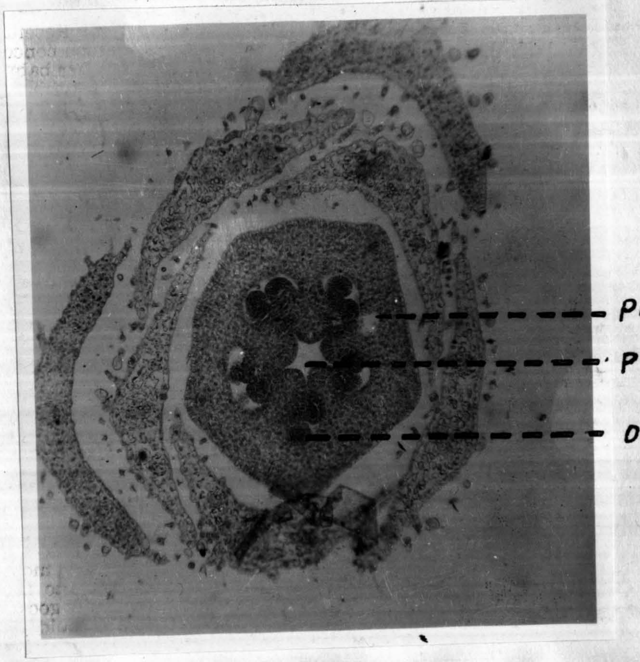

Fig. 24

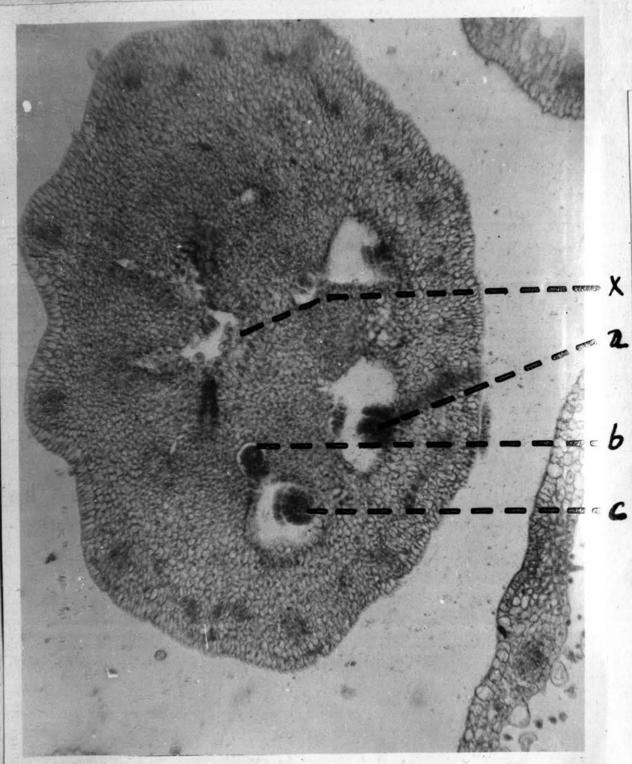

Fig. 25

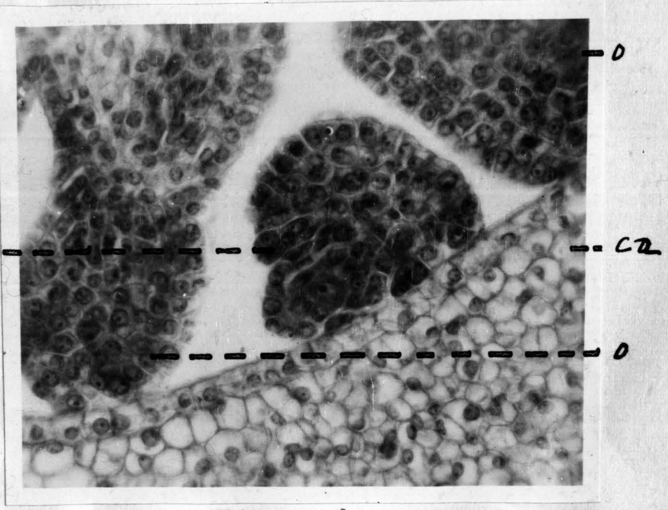

Fig. 26

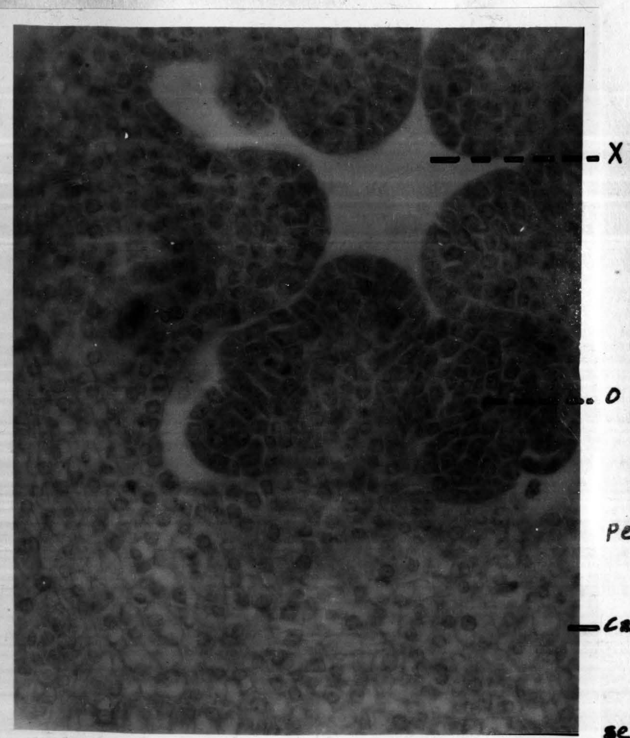

Fig. 27

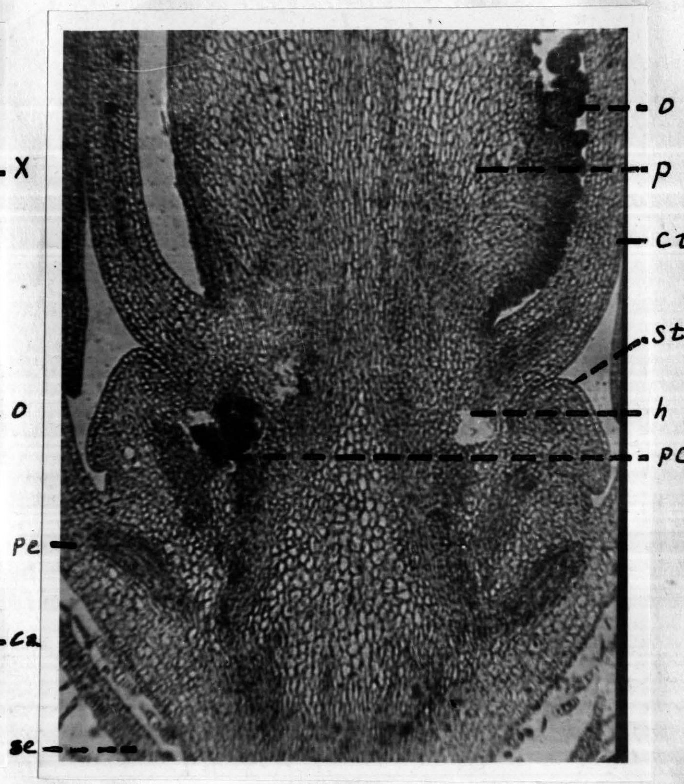

Fig. 28

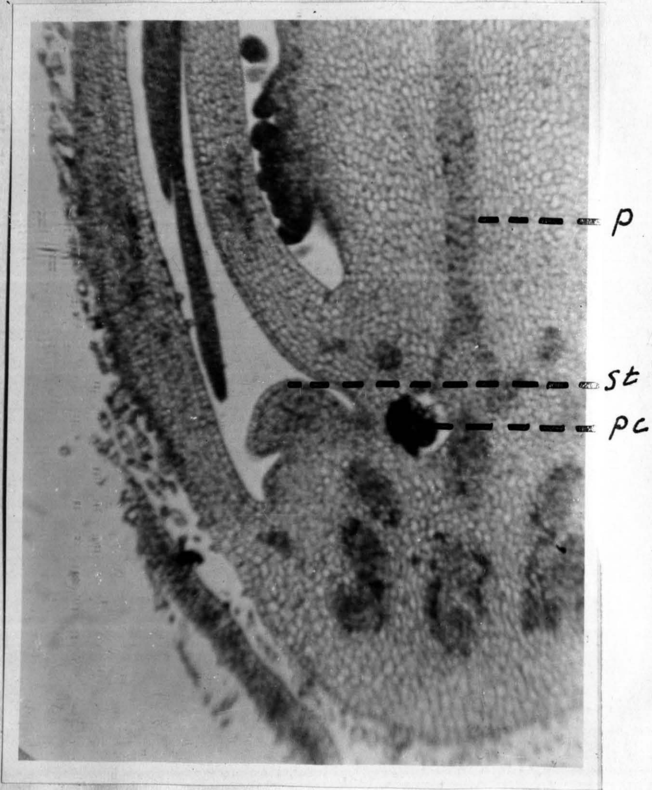

Fig. 29

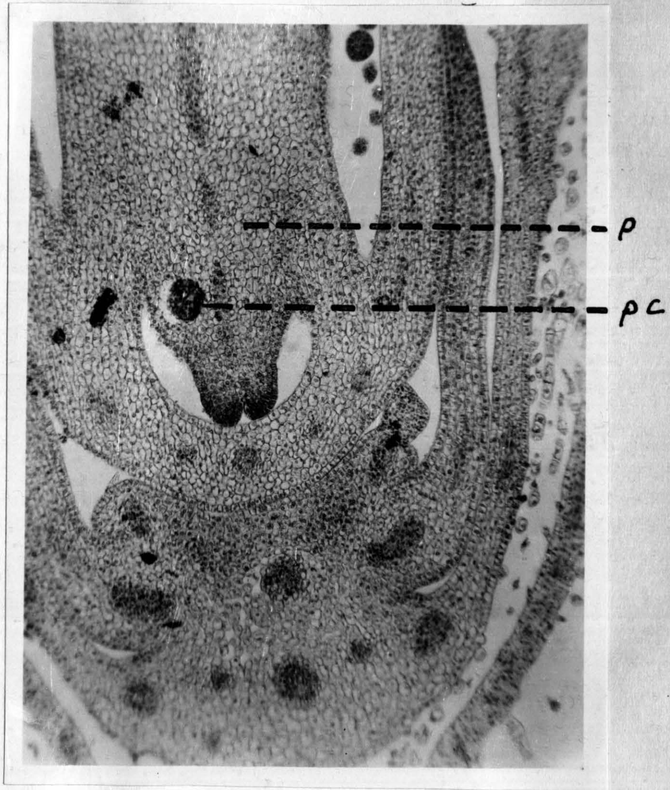

Fig. 30

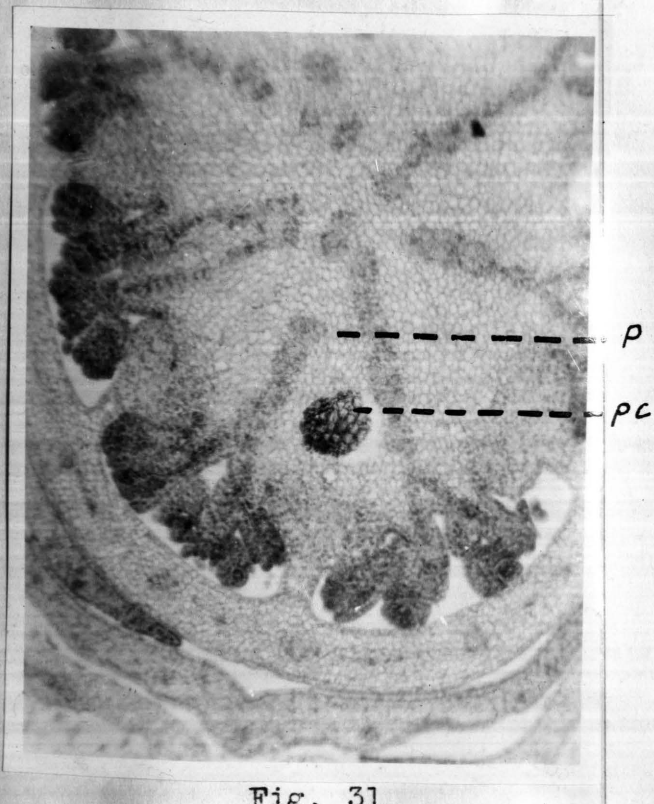

Fig. 31

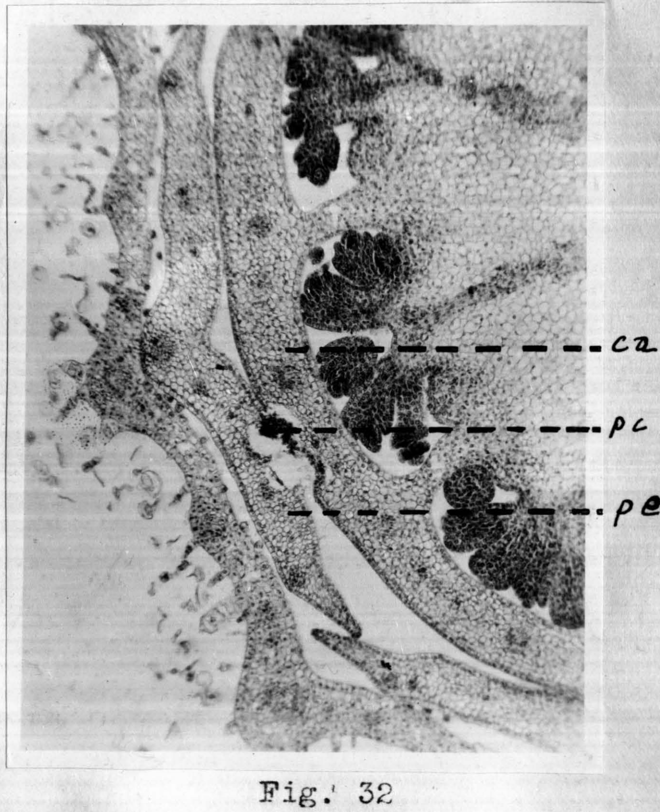

Fig. 32

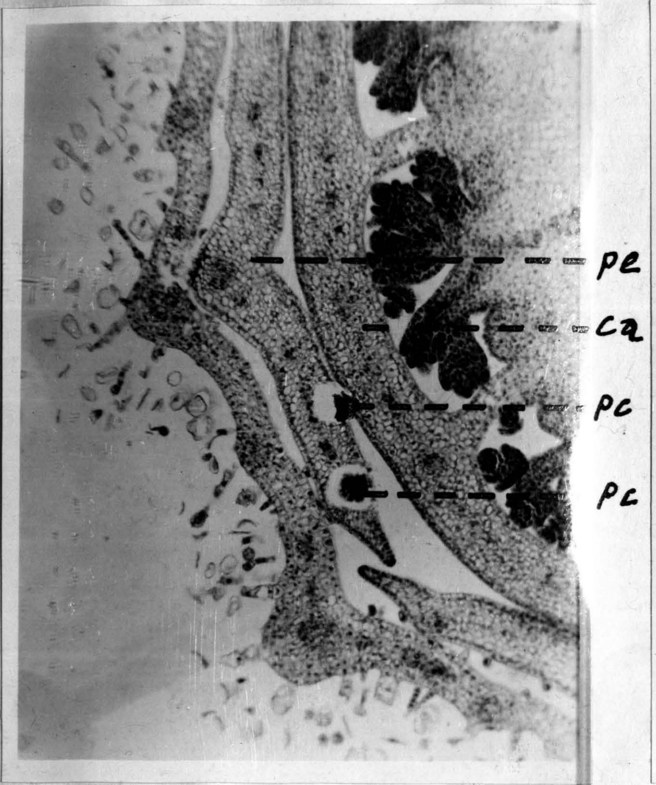

Fig. 33

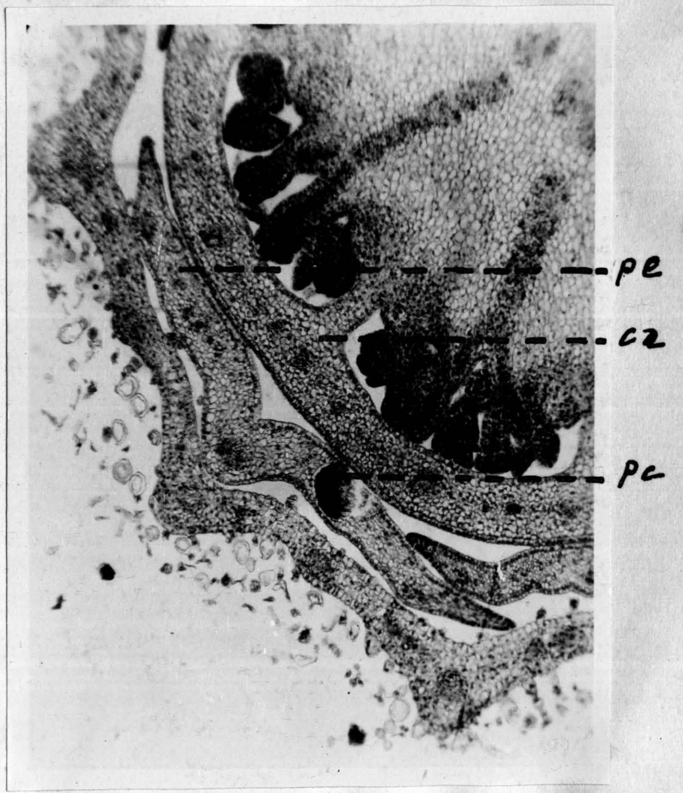

Fig. 34

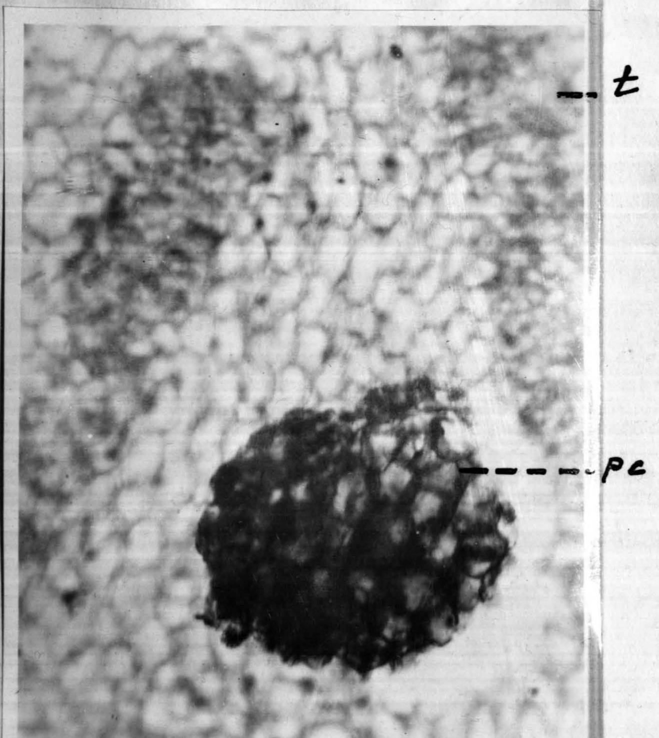

Fig. 35

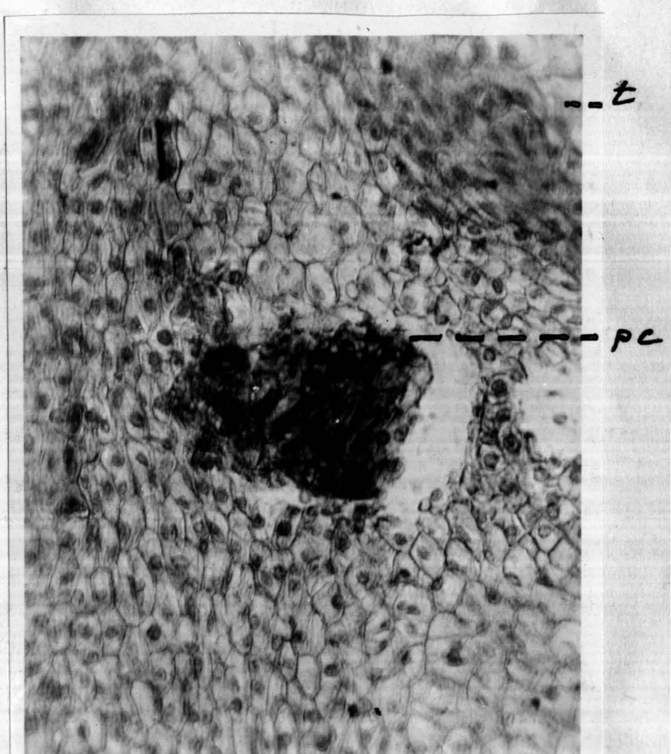

Fig. 36