

EXPERIMENTS IN METHODS FOR THE PREPARATION AND
OF MATERIALS FOR USE IN A COURSE IN "LIVING
PLANT" AS PRESENTED AT THE UNIVERSITY OF
KANSAS.

by

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INTRODUCTION.

The course in "Living Plant" as given at the University of Kansas is an introductory Botany course giving five semester hours college credit. For three days each week, an hour a day is devoted to lectures, demonstrations, and recitations and on each of the remaining two days, two hours is devoted to laboratory work. Each student is equipped with a compound microscope and slides for study, prepared by a technician. It was in connection with the preparation of these slides that the following studies were made.

The course embraces the following topics:

Structure of the Plant Cell.

Living Chloroplasts.

Leaf Structure and Photosynthesis.

Respiration or Energesis.

Conduction of Water and Solutes through the Length of the Plant into the Leaf.

Absorption of Water and Solutes by the Roots.

The Storage of Foods.

Growth.

Cell Division and Plant Reproduction.

The technician's particular problem is the selection of the best materials and their preparation. As the classes are large, numbering more than 225 students, methods must be devised that give uniform, accurate results with a minimum of loss. The question of time is always most important and the permanence of the slides is a factor equally vital. The available material outdoors enters largely at all times into the question of the selection of suitable studies and the ease with which materials can be grown in the greenhouse and kept in satisfactory condition for long periods is another determining influence. It is recognized that too many permanent slides give the student the impression that botany is dead, and far removed from his experiences out of the laboratory. Another drawback to the use of permanent slides is that, in the hands of the students, they are very perishable. Canada Balsam used as a mountant never hardens absolutely

and it gives and springs if the cover-glass is struck by the objective. When the cover-glass is cracked or broken there is no way to salvage the sections.

As a novice in technic, the student will consume too much time and attain indifferent results if left to prepare his own slides. The system of having the slides ready at the beginning of the laboratory period is a device to enable him to have access to excellent material and to get at the heart of the study at once. He is always expected to understand their preparation. This method though saving time for the student means constant experimentation on the part of the technician, and adaptation of the course to the seasons and to the local flora.

SELECTION OF MATERIAL FOR THE STUDY OF THE PLANT CELL.

In the study of the living cell, a large whole cell with nucleus suspended toward its center was sought. A mount sufficiently permanent to be carried over for 24 hours was required in view of the fact that with three divisions of the class lasting almost the entire day, all material had to be ready a day before its use.

ONION. The onion cell, used in so many laboratories was discarded for material showing more prominent

nucleus, plastids, and strands of cytoplasm.

IRIS. (Plates 1 and 2.) After repeated trials with different materials, Iris was chosen as giving the most promise. The white part of the leaves just above the rhizome was stripped of its epidermis with mesophyll cells adhering and these proved excellent. Either the skin on the outside of the leaves or that on the inside is good. This material was used during the early spring before growth had started when the rhizomes were dug up from the frozen ground. In June when the same study was undertaken by another class and Iris prepared in the same way, the results were so poor that another subject has to be selected.

ASPARAGUS. Young, white shoots of asparagus are difficult to peel and do not show nuclei prominently.

CANNA. The white bases of canna leaves show twisted tracheal elements that obscure the epidermal and mesophyll cells.

LILY OF THE VALLEY. The white bases of the leaves of Lily of the Valley can be peeled and the pieces show elongated cells with nuclei near the center. They are fair for study but offer objections because of the small bits of epidermis obtainable.

YUCCA. The bases of Yucca leaves cannot be peeled.

CELERY. Celery cells are small and unsatisfactory because the nuclei are not plain.

SANSEVIERIA. Sansevieria will not peel. Sections of the inner layers in the leaf were imbedded and cut on the microtome. They revealed but few nuclei.

LETTUCE. Lettuce cells are irregular in shape and the nuclei are not prominent.

TRADESCANTIA. Tradescantia stems and leaves cannot be peeled with sufficient ease to make that a good source of material for a large class.

COLEUS. Cross sections of the growing tip of Coleus showed small cells.

RHUBARB. Rhubarb can be peeled for a long distance up the petioles and the epidermal cells are excellent with conspicuous nuclei. It is quite as satisfactory as Iris. The stomata are interesting and very clearly visible.

Fixatives and Stains.

1. Fix in formaldehyde made up with one part of commercial formaldehyde and nine parts of water. The pieces of skin will begin to appear opaque and white when sufficiently fixed. Twelve hours or more is all right but they may stay in formaldehyde indefinitely.

Result: The nuclei were apparent with this fixative and the cytoplasm was granular but no strands were visible. In many of the cells there was plasmolysis.

2. Fix material in absolute alcohol. Then place it in 0.3% HCl. Stain in 0.5 gram of methylene-blue plus 0.5 gram of acid fuchsin in 500 c.c. of distilled water. Rinse in 75% alcohol, mount in castor oil.

Result: The finer distinctions in the cell, like the cytoplasm strands, were not evident with this fixative and the stain was not uniform. There was a tendency toward plasmolysis.

3. Fix in absolute alcohol 3 parts and glacial acetic acid 1 part. Let the material stand in this 6 to 24 hours, and wash in absolute alcohol.

Result: This fixative was discarded because it was impossible to demonstrate strands of cytoplasm and plastids.

4. Mix together equal parts of formalin, pyroligneous acid, and methyl alcohol. Remove from this to 40% or 50% alcohol after 24 hours.

Result: Although the strands of cytoplasm were visible, the other details of cell structure did not appear with sufficient clearness.

5. Fixative. One part 1% chromic acid, plus one part 1% platinic chloride, plus six parts of water. Fix for 24 hours.

Result: With this fixative plastids were demonstrable but the strands of cytoplasm had been altered.

6. Make a saturated solution of carmine in 45% acetic acid; bring to boil and filter.

Result: With this fixative and stain, the material was too deeply colored to reveal the cell structure clearly.

7. Altmann's fixing and staining. Put epidermis strips for 24 hours in a mixture of equal parts of a 5% solution of potassium bichromate and a 2% solution of osmic acid. Then wash for 5 hours in running water. Then place strips in watch glasses for 30 to 60 seconds in a solution of 10 grams of acid fuchsin in 50 c.c. anilin water which is heated to steaming. Then quickly rinse in a mixture of one volume of saturated solution of picric acid in absolute alcohol plus two volumes of water. Finally heat for 30 to 60 seconds in a saturated solution of picric acid in absolute alcohol. Then pass quickly through absolute alcohol, xylene, and oil or Canada balsam.

Result: Repeated trials with this method gave unsatisfactory results chiefly because of the great amount of stain in the walls as well as in the cell contents. There was some plasmolysis.

8. Absolute alcohol as a fixative makes the tissues brittle and somewhat opaque.

9. "Staining Intra Vitam. ---Living protoplasts

may accumulate certain stains from very dilute solutions without injury to themselves. Dahlia, methyl-violet and methylene-blue are particularly suitable for this purpose. Solutions containing 0.001% or 0.002% of the first two stains have given good results in staining living nuclei, while 1 part of methylene-blue in 500,000 parts of filtered rain-water is used for staining living cells containing tannin." (2)

Result: All three of these stains were tried with the conclusion that they could not be depended upon to give uniform results. The methyl-violet gave the most promise but the strips were irregular in the amount of stain absorbed. There was a tendency to plasmolysis.

10. "Stock Chromo-Acetic Solution. Chromic acid 1 gram, Glacial acetic acid 1 c.c., water 100 c.c."(1)

Result: The stock chromo-acetic solution softens the tissues somewhat and causes slight plasmolysis.

11. "Weak Chromo-Acetic Solution. Chromic acid 0.3 gram, acetic acid 0.7 gram, water 99.0 c.c."(1)

Result: Satisfactory results were obtained with this fixative. Our experiments justified the confidence that has been placed in this dependable fixing fluid.

12. Flemming's Fixative: One per cent chromic acid 180 c.c. plus 2% osmic acid 25 c.c., plus glacial acetic acid 12 c.c. plus distilled water 210 c.c. Fix for 48 hours; wash in running water half day or over night. The osmic acid solution must be made with extreme care according to the directions given in Stevens' "Plant Anatomy" page 259.

Result: The results obtained with Flemming's fixative were so superior to all the others tried, with the single exception of the weak chromo-acetic solution, that it was adopted in all the subsequent work. None of the other fixatives retain the delicate texture of the protoplasm and the normal position of the nuclei and plastids. There is no difficulty with shrinkage in Flemming's fixative. The only drawback to its use is the expense for the osmic acid but since every subsequent step in the preparation of slides is dependent on preserving the material with the niceties of detail apparent, any economy for a cheaper fixative is deemed unwise.

13. Stain: 3 gram eosin, 0.2 gram aniline blue, 100 c.c. distilled water. Stain for 24 hours; wash in water then in 95% alcohol, then pass through acid alcohol.

Result: Excellent results were obtained in using this stain following fixing with Flemming's.

The nucleus was red and the protoplasmic strands, blue. Plastids and nucleoli appeared in sharp contrast to other parts of the cell.

14. Stain. Place material in 1% aqueous fuchsin solution, wash with water, then stain in 0.2% aqueous solution of methylene-blue. Rinse in alcohol; mount in castor oil.

Result: This stain gave results inferior to those obtained with the preceding stain. A nicer contrast of color was obtained with the eosin and aniline blue.

15. Delafield's Haematoxylin used as a stain on the Iris epidermis gave a violet color to the cell wall but as the object of the study was to see the protoplasm as little obscured as possible by the cellulose inclosing it, this stain was not employed.

Permanent slides are desirable for study of the cell, but semi-permanent slides can be utilized. Pieces of epidermis should be thoroughly washed of the fixative and mounted in a solution of saturated chloral hydrate 1 part, iodine 1 part, water 1 part with enough glycerine added to make the mixture a 3% glycerine solution to keep them from drying. If no stain is desired a mounting fluid of saturated chloral hydrate 1 part and water 2 parts with glycerine added as above is satisfactory.

LIVING CHLOROPLASTS. (Plate 3.)

Sections of *Sansevieria* leaf were chosen for this study because the cells are large and contain relatively few chloroplasts. It is possible to make measurements accurately. A freezing microtome cuts thin sections but the ordinary microtome can be used if the piece of leaf to be cut is clamped into the holder with its edge near the knife. The softer mesophyll tissues are supported in this way by both epidermises. The sections can be mounted in dilute glycerine. (3% to 5%).

LEAF STRUCTURE AND PHOTOSYNTHESIS.

For a critical study of the leaf in cross section, permanent slides are most satisfactory. We tried several subjects, *Melilotus alba*, *Salvia splendens*, *Populus deltoides* and *Syringa vulgaris* and settled upon the last as most suitable. The *Populus* leaf shows a double row of palisade cells and it is so full of gums and resins that it is almost impossible to stain. The *Melilotus alba* has a thin epidermis that shows a tendency to tear off in sectioning. *Salvia splendens* is excellent. It has interesting epidermal glands and hairs. Potato leaves are simple in structure and easy to imbed. The method of preparation described was used with

Syringa. With the other leaves chromo-acetic fixative instead of Flemming's was used which might account in a measure for the superior result obtained with Syringa. (Plate 5.)

The leaves were cut into small pieces about 2 m.m. by 4 m.m. and killed in Flemming's fixative. The air was pumped out with a suction pump. The directions given by Professor Stevens in his "Plant Anatomy" pages 259-263 for The Hardening Process, Imbedding in Paraffin, Sectioning Material Imbedded in Paraffin and Mounting Paraffin Sections were followed out in detail. The results were very satisfactory and it was possible to cut sections at 5 mikrons. The great difficulty with leaves is due to the shrinkage that is likely to occur because of the large air spaces. Our experience suggests that with the selection of the best leaves and the use of Flemming's fixative, this difficulty is overcome. Pumping out the air is an important detail that cannot be slighted.

A modification of Pianeze IIIb, a stain, devised for use in the differential staining of fungous and host cells, was used on the leaf sections. Malachite green 1.35 grams, acid fuchsin .45 gram, "Martius gelb" .05 gram, distilled water 150 c.c., alcohol 95% 50 c.c.

For use with plant tissues the procedure is as follows: After fixing on the slide sections cut from material imbedded in paraffin, dissolve the paraffin in xylene and rinse in 95% alcohol, stain in the undiluted mixture over night; remove excess stain in water, and decolorize in 95% alcohol, to which a few drops of hydrochloric acid have been added. Clear with a carbol-turpentine mixture, remove clearer in xylol and mount in balsam.

In a search for a substitute for balsam Valspar was used. It may be dropped from a small brush on to the sections, where it spreads out thinly and dries very hard. The cover-glass may be dispensed with entirely with thin sections like those of leaves or root tips. For thicker sections like stems, Valspar may be used to replace balsam as a mountant with the cover-glass. It is much less satisfactory used in this way because it is only at the edge of the cover-glass where it is exposed to the air that Valspar dries hard. The advantage of Valspar used with a cover-glass is that the slides may be washed. Great care is necessary to keep them away from dust while they are drying.

A rapid method of cutting sections of a leaf is described by Chamberlain. "Lay one leaf on another until you have a bundle of them which will be nearly square in transverse section. Wrap the

bundle with string for about 15 m.m.; cut the bundle transversely so that about 5 m.m. of the bundle will project beyond the tied portion. Dip in melted paraffin, fasten the tied portion in the sliding microtome, and cut with the knife placed obliquely."

These sections may be mounted in 3% to 5% glycerine, using a camel's hair brush to handle them.

The procedure in preparing slides to show starch grains in the chloroplasts was as follows: A branch of lilac was covered with a black cloth for two days or more to insure complete destarching of the leaves. Then pieces of the leaves were imbedded as in the former study. A ribbon of section cut from destarched leaf and a ribbon of sections cut from a leaf picked in the sunshine were mounted on the same slide. The preparation of the slides up to this point may be completed several days to a week before they are needed.

Two methods of staining and mounting were used with success.

1. a. Cut off the paraffin in xylene.
- b. Rinse in 95% alcohol.
- c. Stain in chloral hydrate 1 part, iodine 2 parts.
- d. Rinse in saturated chloral hydrate in 95% alcohol to which has been added some crystals of iodine.

- e. Mount in castor oil 5 parts, saturated solution of iodine in 95% alcohol 1 part.
- 2.
- a. Cut off the paraffin in xylene.
 - b. Rinse in 95% alcohol.
 - c. Use a mounting fluid made in the proportion of chloral hydrate saturated in 1% acetic acid 32 c.c., potassium iodide iodine 64 c.c., glycerine 4 c.c.

These reagents were selected as a result of a series of experiments with chloral hydrate and iodine used in different proportions. The chloral hydrate has a tendency to dissolve the chloroplastids and make them too transparent and the iodine has a tendency to make the starch grains too dark. We wished to be able to distinguish starch grains within the chloroplasts.

Another series of experiments resulted in the selection of the reagent, 1 part of saturated iodine in alcohol and 5 parts of castor oil. The iodine was used in different proportions with castor oil, phenol and Cedar oil. No iodine stain is permanent but the iodine-castor oil combination stains and preserves the material for several weeks.

A striking supplementary study of starch within the leaf was found by using leaflets of Sword fern. Destarching of the guard cells is accomplished very slowly, three days or more being required.

Leaves taken from plants in the dark and plants in the light were treated in the same way.

- a. Bleach in 95% alcohol.
- b. Stain in saturated chloral hydrate 1 part, potassium iodide iodine 2 parts.
- c. Dehydrate in chloral hydrate saturated in 95% alcohol to which has been added some crystals of iodine.
- d. Mount a leaflet with starch and one without starch together in castor oil 5 parts, iodine in alcohol 1 part.

Stomata. (Plate 6)

Loosen the epidermis of an Iris leaf over the round of a chair so that it will strip off easily. Put into 70% alcohol. When needed, rinse in 95% alcohol. An easy way is to handle a quantity of the material in a small bottle. The 70% alcohol may be poured off and 95% added. This in turn may be poured off and a solution of saturated chloral hydrate 1 part and iodine 2 parts added. Dehydrate with chloral hydrate saturated in 95% alcohol, to which a few crystals of iodine have been added. Mount in castor oil 5 parts, saturated solution of iodine in 95% alcohol 1 part. Pieces of epidermis dry very quickly after being rinsed in 95% alcohol and must be handled carefully to keep

free from air bubbles.

Whole leaves of Wild Smilax bleached with alcohol and cleared in chloral hydrate show the stomata. The advantage in using Iris epidermis is due to the fact that with the starch in the guard cells stained brown, the stomata are brought out in a striking way.

Venation. (Plate 4).

The study of venation is accomplished by using a whole leaf bleached in 95% alcohol and cleared in saturated chloral hydrate. A satisfactory mounting fluid is 20% glycerine, 40% saturated chloral hydrate and 40% water. There are doubtless many leaves that would answer well but we found flax as satisfactory as any. Barberry shows typical venation and clearly defined palisade tissue. *Spiraea Thunbergii* has very small palisade cells, but the venation is excellent.

CONDUCTION OF WATER AND SOLUTES THROUGH THE LENGTH OF THE PLANT AND INTO THE LEAF.

Permanent slides with a section of Smilax, which is a mono-cotyledon and *Aristolochia*, which is a dicotyledon, were used for a study of vascular bundles. The Smilax was desilicified before sectioning, according to the method described by Stevens, page 297. Smilax was chosen because of the excellent sections

obtainable. Cane or corn can be used. The sectioning was done one day and the staining accomplished over night. A great many of these sections were stained in a small vial. Safranin was chosen as a single stain giving good contrast in the tissues. At the same time the Smilax was being stained, vials of Aristolochia sections were stained also. After remaining in the stain all night, the sections were emptied out in a watch glass and rinsed in 95% alcohol. Two or more alcohol rinses were required before dehydration was complete, after which the sections were transferred to xylene. The rinsing was accomplished as rapidly as possible to prevent excessive destaining, but the sections remained for some time in the xylene. They were handled easily with a camel's hair paint brush. A section of Aristolochia and one of Smilax put on the same slide were mounted in balsam. As clothes pins were used to clamp the cover-glasses in place, the excess of balsam was squeezed out and the thin layer remaining hardened more rapidly and completely. They remained clamped for several weeks.

"To show that water and solutes from the soil rise through these bundles place stems of garden balsam (white flowered variety is best) upright in a weak solution of safranin. The path of ascent will

be red." It is not possible to use permanent or even semi-permanent slides for this study as the stain diffuses through the tissues very rapidly. If a piece of garden balsam stem is put into the stain it will be ready to section in about 7 minutes, when the tracheal elements only show the red color. The sections mounted in dilute glycerine (4%) are good for a few hours. A drop of glycerine added to the side of the cover-glass will keep them longer.

A study to illustrate the continuity of the veins and vascular bundles was devised by taking the growing tips of flax and removing about half of the leaves. The stems with some leaves attached were bleached in alcohol and cleared in chloral hydrate. They were then mounted in 20% glycerine made up with 40% saturated chloral hydrate and 40% water. The veins were visible in the leaves and in the stem as well as a little below the growing tip, the small rudimentary leaves and apex of the stem showing no differentiation of tissue.

THE STORAGE OF FOODS.

In all the food storage studies use was made of semi-permanent slides. Because iodine is a temporary stain and the only useful stain for starch

the semi-permanent slide with castor oil as the mounting medium was devised.

The Irish potato comes to mind first for a demonstration of starch. Thin sections of potato may be cut on the microtome. After these are dehydrated thoroughly in 95% alcohol they are ready to mount in castor oil and iodine. Mounts made with the castor oil-iodine will keep for weeks if they are protected from dust. The oil has a tendency to creep over the edge of the cover-glass but this is a minor objection.

Sweet potato is an excellent alternative study if one wishes to vary the course from semester to semester. (Plate 12). Acorn soaked in water and then cut on a microtome gives a fine view of starch grains somewhat smaller than the potato. (Plate 11). Carrot shows a few small sharply defined starch grains. Peanuts of course used raw, can be soaked in water and cut thinly to demonstrate starch and protein. (Plate 13). Lima beans, pinto beans and scarlet runner beans are all large enough to section to advantage and they show excellent starch and protein content. Sections of sunflower seeds treated with the castor oil-iodine reagent make an interesting protein study.

For the study of oil, Sudan III was applied

to sections of seeds like castor bean, peanut, walnut and sunflower. (Plate 14). The castor bean seeds are too small and soft to cut on the microtome but the others can be sectioned nicely. The sections can be put into small vials of Sudan III and left over night or longer. They can be mounted with a camel's hair paint brush in the stain or in a dilute glycerine-alcohol solution. They keep several days if protected from dust. Aleurone grains are best demonstrated in castor bean, or walnut, or Brazil nut by removing the oil present with chloroform. The castor oil-iodine mountant gives a fine differentiation in the structure of the aleurone grains. (Plates 15 and 16). An excellent substitute stain is made with 1 part of concentrated alcoholic solution of fuchsin and 10 parts of castor oil. These two stains were selected from a series of experiments with stains including eosin, anilinelb, Bismark brown, magdalarot, anilinrot, bleu de Lyon, fuchsin, dahlia and nephtylamingelb.

Starch stored in stems is well demonstrated in the grape if the material is gathered in the late summer or autumn. It can be preserved in alcohol-glycerine and water, equal parts, and cut at any time. The iodine-castor oil mount gives a fine differentiation though the student needs to be cautioned about the yellow

that appears in the lignified tissues. The protein that may be present in the phloem is a reddish-yellow within the cell cavities but the lignified tissues of course are cell wall. Photographs do not give the nice distinctions in the sections because the brown of the starch and the yellow take alike.

As substitutes for grape, many stems might be mentioned. Trumpet creeper cut fresh in June and stained in iodine is excellent for the starch sheath but indifferent for other storage. It would no doubt be better at another season. Magnolia cut at 25 mikrons gave a pretty demonstration of storage starch. (Plate 18). Hops cut fresh in June showed food in storage as well as smaller starch grains in the green tissues of the cortex. (Plate 19). The stems of *Menispermum Canadense* have interesting structure and they give a most excellent substitute for grape to illustrate storage tissues. (Plate 24). *Rhus cotinus* is another shrub that gives a good idea of the storage tissues of stems.

Slippery Elm bark. Mucilage may be found in abundance in the inner bark of *Ulmus fulva*. It is interesting to study in connection with reserve food although its use as food is doubtful. It is always present and for that reason may be collected at any season. The druggist can supply slippery elm but

it is better if taken fresh from the tree. The chief difficulty presented by the preparation of the material is due to the fact that the mucilage dissolves in water. It will swell and form a slimy mass about the sections unless it is first fixed in lead acetate. This is best accomplished by placing pieces of bark in a 10% solution of lead acetate and pumping out the air. They should be left over night in this solution and then washed thoroughly in running water. Then they can be transferred to a solution of alcohol 60% and glycerine 40% and kept indefinitely. When needed the bark should be cut on a sliding microtome at about 35 mikrons. If thinner sections are used the hardened masses of mucilage have a tendency to fall out and are lost in staining.

Stains. Methylene-blue is a good mucilage stain but not adapted to use in permanent slides. (Plate 17). If the sections are placed in a deep blue aqueous solution of methylene-blue in equal parts of alcohol, glycerine, and water they will absorb sufficient stain. They should stay in the stain over night or longer. From this they can be mounted in dilute glycerine. (5% to 10%).

Congo Red is recommended as a mucin stain. Tests carried on with it were not satisfactory because the stain disappeared from the sections when

they were mounted.

Picric acid was tried in aqueous solution as well as alcoholic solution but gave inferior results.

Bismark Brown, also known as Vesuvin, gave most excellent results in the preparation of permanent Slippery Elm sections. Bismark Brown is a powder soluble with difficulty in water. One gram dissolved in one hundred cubic centimeters of boiling water gives a stain of sufficient intensity. It should be filtered. Although Bismark Brown stains rapidly it does not overstain. A number of sections can be stained at once. The mucilage takes the stain with avidity and the cellulose of the phloem takes a different hue. Starch that may be present appears almost black. After the staining, the sections may be rinsed in two waters. This is easily done by lifting the sections out of one watch-glass and transferring them to another with a small camel's hair paint brush. They are then ready to be mounted one at a time in lactophenol gum.

Lactophenol.- Carbolie acid crystals 20 grams, Lactic acid (sp. gr. 1.21) 20 grams, Glycerine (sp. gr. 1.25) 40 grams, Distilled Water 20 grams.

Lactophenol gum for mounting medium.- Dissolve 38 grams of pure gum arabic crystals in 50 c.c. distilled water; add 5 grams of glucose and 6

grams of lactophenol; filter through glass wool. It is to be used cold.

This mounting medium used with the Bismark brown mucin stain was successful in preserving in permanent form the sections of Slippery elm. Lactophenol gum is used only with acid stains. It draws out enough of the stain to make a halo about the material, but it is transparent and clear and hardens thoroughly. Great care must be taken in mounting the sections to see that they are free from air bubbles as these become imprisoned in the rapidly hardening gum.

GROWTH.

The study of growth is approached by observation of different types of seedlings. Lima bean has a large seed but the moulds which attack it during germination often interfere with its successful growth. This need to cause no anxiety because so many equally good seeds are available. Pinto bean and scarlet runner bean and squash and sunflower and walnut and peanut might be mentioned as some of the possibilities. Castor bean seeds illustrate a type with large endosperm and they are always easily obtainable. We use corn and date for monocotyledons. The dates germinate slowly

but their vitality is good. The growing of the seedlings offers no problems of difficulty for the technician. The study of growing stems is illustrated by using buds in winter condition. Large protected buds are found on the horse chestnut and hickory and sycamore and cottonwood and poplars. Interesting naked buds occur on the Sumac and Ailanthus. If these are brought indoors during the winter, they will swell and open. Several flower buds that open similarly are available anywhere. Examples of these are the peach and Japanese quince. Lilac buds are interesting leaf buds.

The study of buds is accompanied by a study of the secondary thickening of stems. For this work sections of one and four year old *Aristolochia* are possibly best. (Plates 7, 22, and 23.) *Menispermum Canadense* which is widely distributed offers a good alternative study. Though the years of growth are not so clearly defined in the *Menispermum* stem, the vascular bundles are sharply outlined. (Plates 24 and 25.) Temporary slides may be quickly made by floating a number of sections in a watch glass of water to which a drop of safranin has been added. The lignified tissues absorb the stain eagerly. With 20% glycerine 40% saturated chloral hydrate, 40% water as a mounting medium, the sections will keep for a time. Permanent slides do not offer new obstacles to the technician.

We used the Pianeze IIIb stain similar to that employed in making leaf sections: Malachite green 0.50 gm., acid fuchsin 0.10 gm., "Martius gelb" .01 gm., distilled water 150 c.c., alcohol 95% 50 c.c. The directions for staining and clearing are given on page 13.

Haematoxylin used with safranin or erythresin gives a pretty permanent mount. The chief difficulty is always in cutting good sections and a mastery of the microtome is essential.

Growth in the monocotyledonous stems is illustrated by a section of either young corn or cane from the first internode above the permanent root system mounted on a slide with a section of an older stem from the same internode. (Plates 20 and 21.)

There is nothing to be gained in having these slides made fresh and permanent slides stained as the dicotyledonous stem slides are stained, are useful. Cane has to be desilicified like Smilax (page 17) and hardened in alcohol before sectioning.

We found the best way to handle stem sections in staining was to use watch glasses and to pour off most of the liquid above the sections and to draw off the remainder with a pipette. The sections were handled rapidly with a small paint brush.

CELL DIVISION AND PLANT REPRODUCTION.

For the study of cell division, permanent slides of onion root tip or Mayapple root tip offer the best solution. (Plates 28 and 29.) The triple stain method described by Professor Stevens on page 263 of his "Plant Anatomy" was followed in detail to the final step. Here instead of "placing a drop of Canada balsam toward one end of the group of sections and lowering a cover-glass over it" we let a drop of Valspar spread out thinly over the sections. It hardened in a day or two and offered perfect protection to the sections without the use of a cover-glass.

Yeast can be grown easily at any time and temporary slides can be made so rapidly that there is little advantage in any other method. Liquid yeast: To a cup of potato water, add a cake of compressed yeast and a measuring spoon of sugar. If the yeast is started in the evening, it will be dividing rapidly by the next morning. One small drop mounted under a cover-glass will be teeming with dividing colonies. (Plate 27.)

Pleurococcus is abundant everywhere. If some pieces of bark on which it is growing are immersed in water over night and then put in diffuse light under a bell jar for a day they will be brilliantly green.

A tiny mass of this green lifted by a dissecting needle and mounted in water makes a temporary slide that is prepared in a very short time and is excellent for study. (Plate 28.)

The course is completed with a study of flowers and here the limits of available material are boundless. Large waxy flowers like tulip or trumpet creeper or Yucca or Narcissus are excellent as introductory types. The work is done without the compound microscope and the technician is relieved of preparing slides.

CONCLUSION.

We were impressed throughout this study, with the relatively few reagents we found most serviceable. For the purpose of the course in "Living Plant", less than a dozen stains are needed and only about two dozen chemicals. These include the commoner things like alcohol, glycerine, xylene, chloroform, etc. The reagents worked out for one study were useful in several. We found that chloral-hydrate iodine in the proportion of one part of saturated chloral-hydrate and two parts of a potassium iodide iodine solution was indispensable among the reagents. Castor oil five parts, saturated iodine in 95% alcohol one part, justified itself as a stain and mountant in

many studies. Another useful combination for mounting was 20% glycerine, 40% saturated chloral-hydrate, 40% water.

A field as yet only partly explored is in the selection of the best materials. This is the feature that makes such a course flexible and changing the material often, works to the advantage of those in charge. The technician has at his command, all the resources of the out-of-doors and this is his inspiration for continued investigations.

ACKNOWLEDGMENT

The writer is grateful to Professor W. C. Stevens and Professor Charles M. Sterling of the University of Kansas for their assistance throughout the preparation of this study.

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2. Stevens, W. C. Plant Anatomy, P. Blakiston's Son & Co. Philadelphia, 1924.
3. Vaughan, R. E. A Method for the Differential Staining of Fungous and Host Cells. Annals of the Missouri Botanical Garden, May, 1914.

PREPARATION OF THE PLATES.

The following plates are photographs of slides prepared for use in the "Living Plant" classes. They illustrate the type of material that is put into the hands of the students for their study.

The essential features of the photomicrographic apparatus consisted of an Edison "Pointolite" electric lamp and a projection microscope with tube set horizontally. The eyepiece of the microscope was connected by a close fitting collar to a camera, made by a bellows, so that the image was thrown directly on the ground glass at the back. The microscope was equipped with three objectives, one low and two medium power and one medium and one high power eyepiece, with the different combinations of which, a magnification of from 60 to 570 diameters was obtained. The image was brought to focus on the ground glass by the coarse and fine adjustments of the microscope. When a sufficiently sharp image was obtained, a plate holder carrying the sensitive paper held in place by a plain glass, was slipped into the position of the ground glass. The exposure was made directly upon developing paper resulting in a negative print. The developing was accomplished as with an ordinary print by using a developing fluid, followed by hypo, and subsequent thorough washing and drying. For the developing a dark room was required but

by drawing the curtains and cutting off all light possible, the work was accomplished with a ruby light in the daytime. Most of the pictures were made on "Haloid Rito", a developing paper not very sensitive. Although it required a long exposure varying from one minute for some slides to eight minutes for others, the danger of fogging in handling was slight. For a few of the slides a more rapid paper was found necessary and Cyco enlarging paper, a developing paper about 30 times as rapid as the preceding, was chosen. The whole apparatus was mounted so that it shook as people moved about in the rooms above and through the hallways making the more sensitive paper necessary in some cases to do away with a too long exposure and too great danger from jarring.

The slides were prepared as for use in the classroom. The contrast furnished by staining was a detail lost in reducing to black and white. Of course the negative is the opposite of what we are accustomed to see in text books, the back ground appearing black and the figures white.

Each step in making these photographs was exacting. A clear slide free from air bubbles and other imperfection was the first requisite. The matter of obtaining a sharp focus was more difficult than would appear at a glance, because of the thickness of the

tissues. Where detail in one direction was desired frequently detail in some other direction had to be sacrificed. The time for the exposure was determined in each case by experiment.

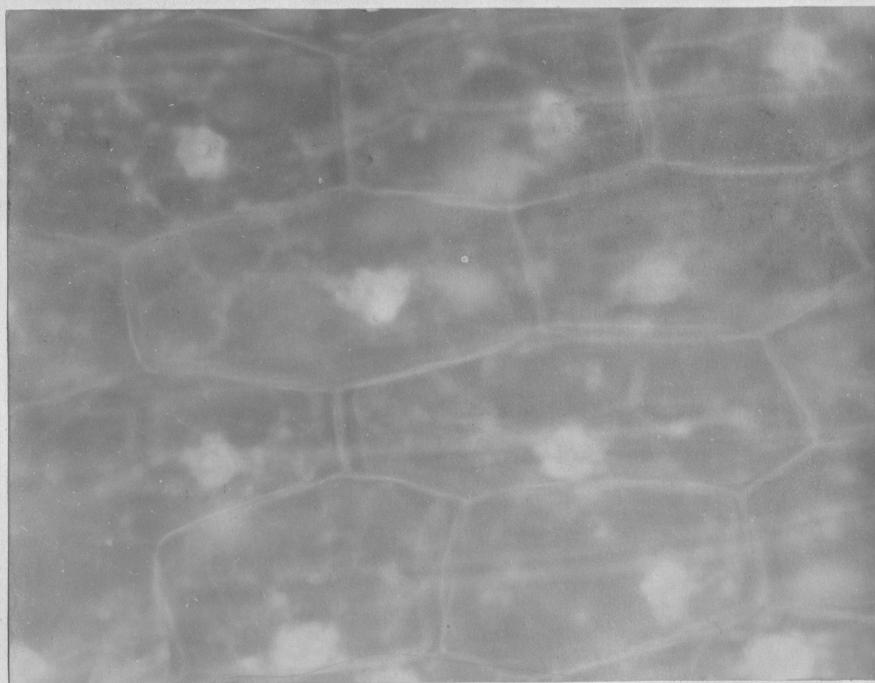
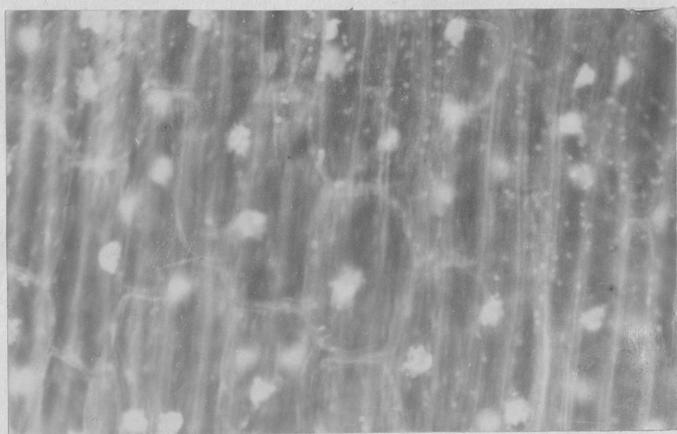


Plate 1. Mesophyll cells of Iris. x270.
Fixed in Flemming's fixative, stained in eosin and
aniline blue. Exposure 5 minutes.

Plate 2. Cells of Iris leaf. x570.
Fixed in Flemming's fixative, stained with fuchsin
and methylene blue. Exposure $\frac{1}{2}$ minute, sensitive
portrait paper.

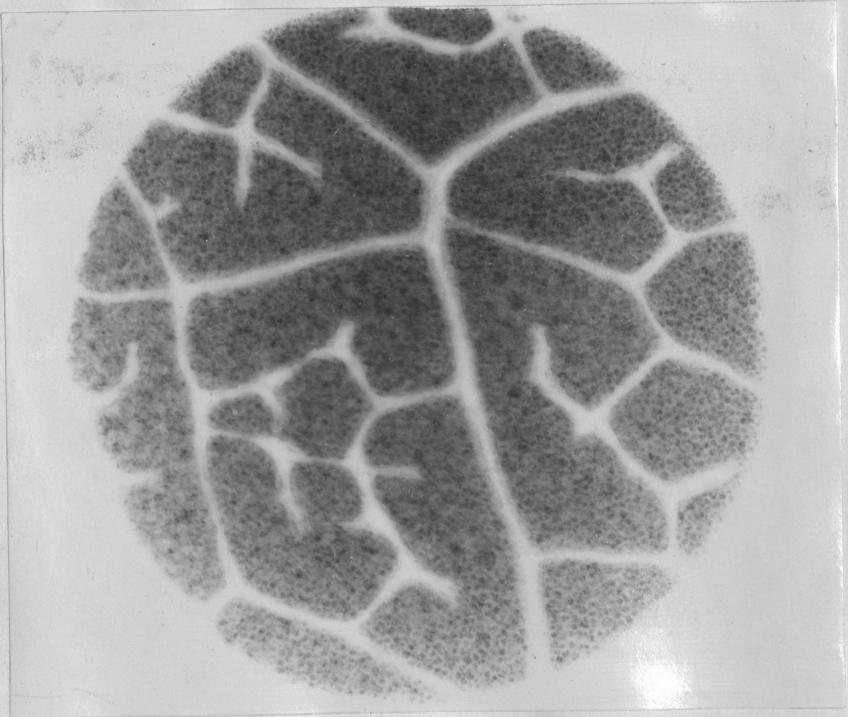
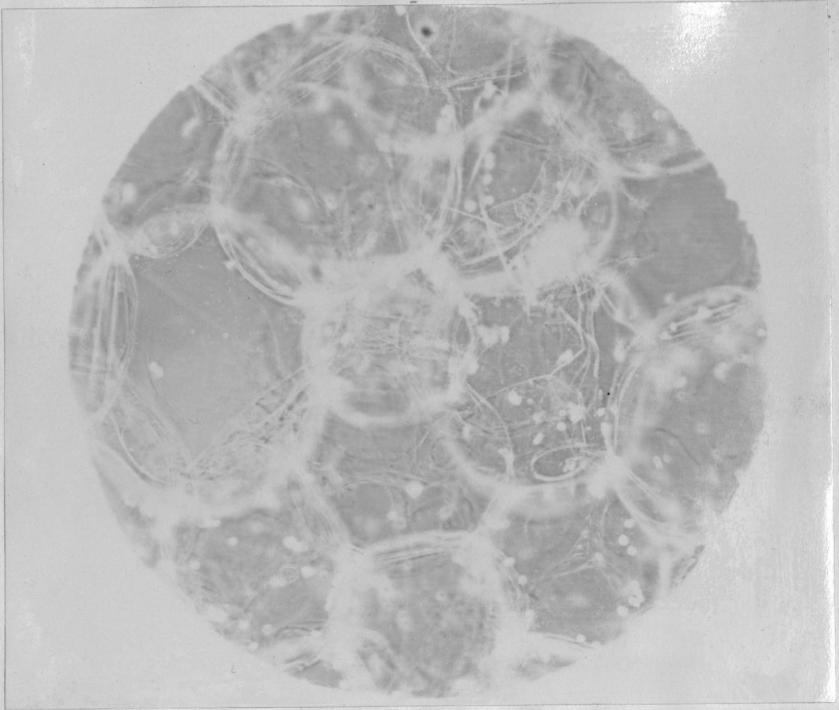


Plate 3. Mesophyll cells of Sansevieria showing scattered chloroplasts. x270.

Plate 4. Venation of Barberry leaf. x60. Whole leaf bleached in chloral hydrate, showing the palisade cells. Exposure 3 minutes.

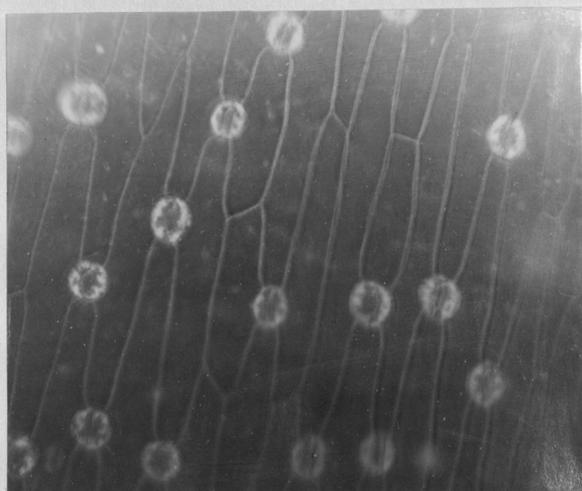
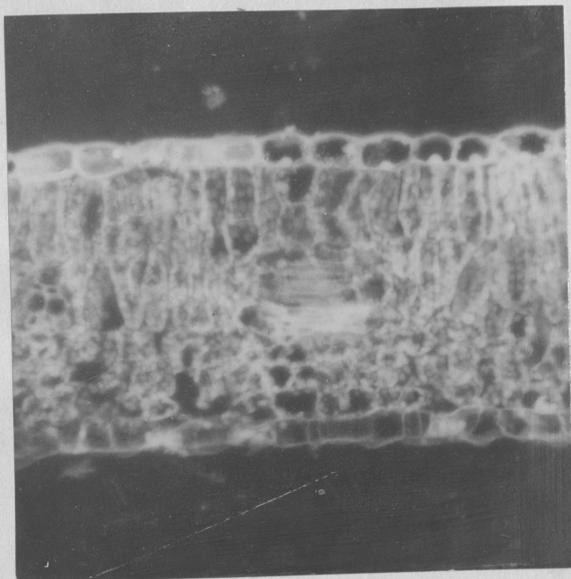


Plate 5. Section of Lilac leaf. x270.

Flemming's fixative, Pianese IIIb stain, mounted in
Valspar. Exposure 7 seconds on rapid portrait paper.

Plate 6. Iris epidermis with stomata. x60.

Cleared in chloral hydrate, mounted in castor oil-
iodine. Exposure 5 minutes.

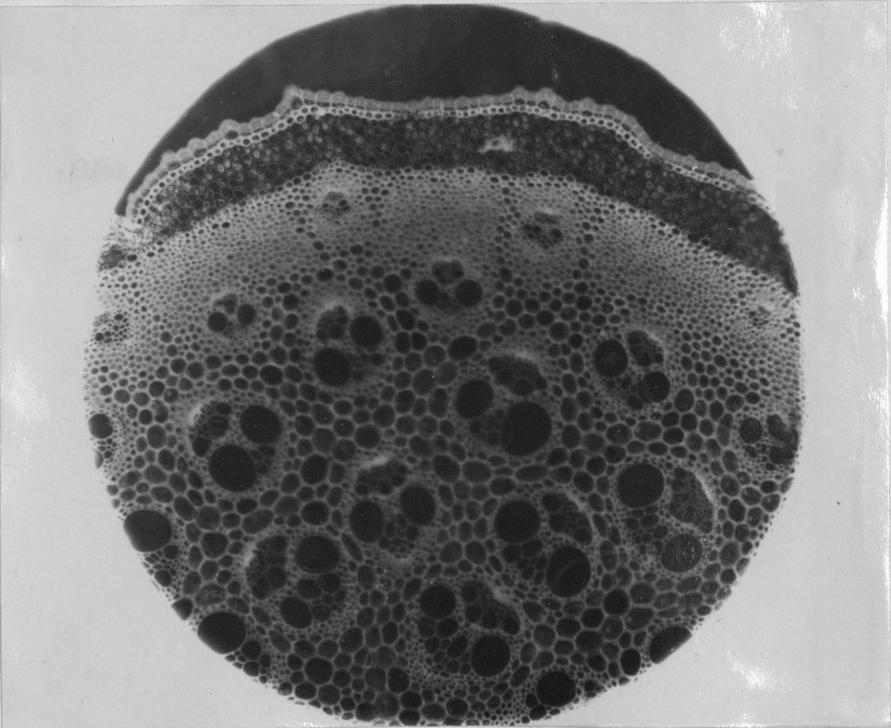
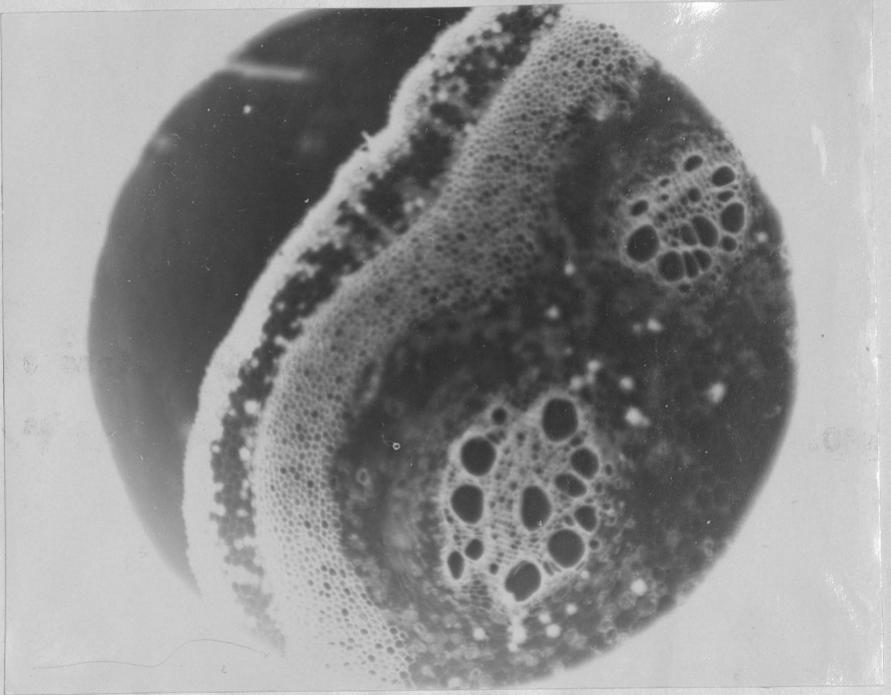


Plate 7. Young *Aristolochia siphon* stem.
x60. Stained with Pianese IIIb, mounted in balsam.

Plate 8. *Smilax ovata* stem. x60. Stained
in safranin, mounted in balsam.

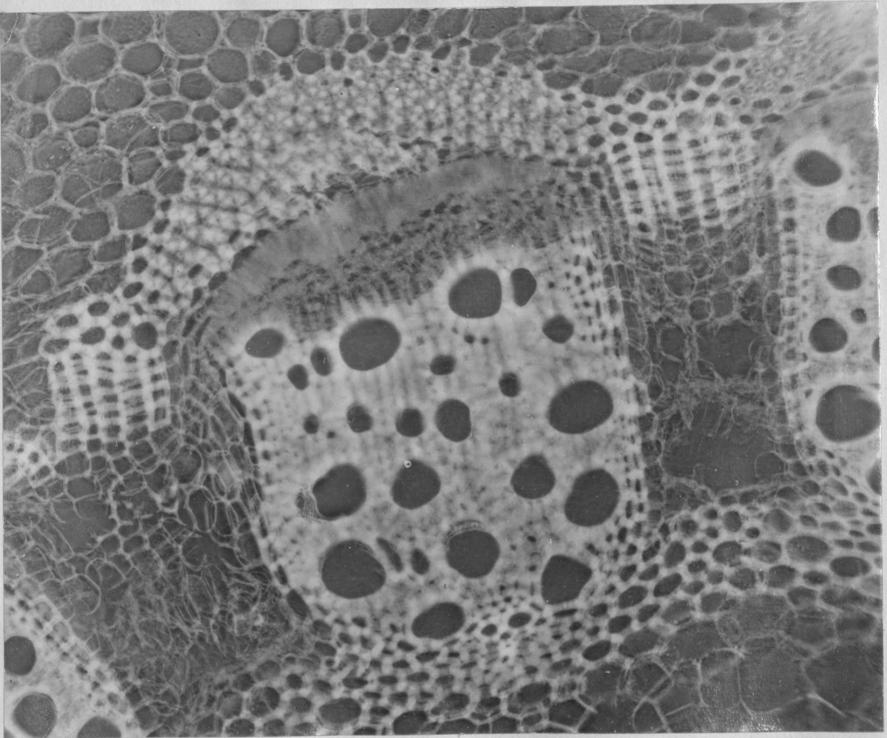
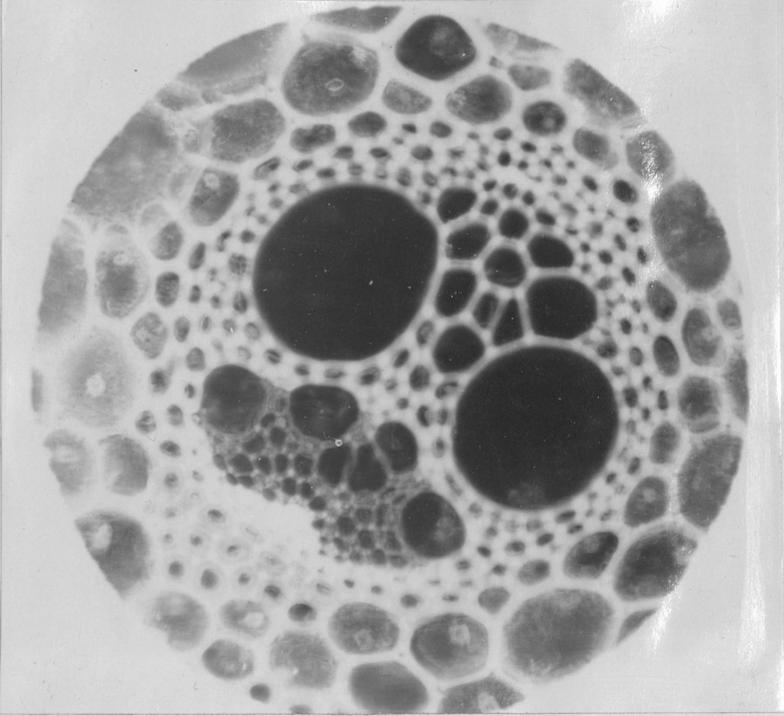


Plate 9. Monocotyledonous vascular
bundle, Smilax. x270.

Plate 10. Dicotyledonous vascular
bundle, Menispermum Canadense. x125. Cut fresh,
mounted in chloral hydrate.

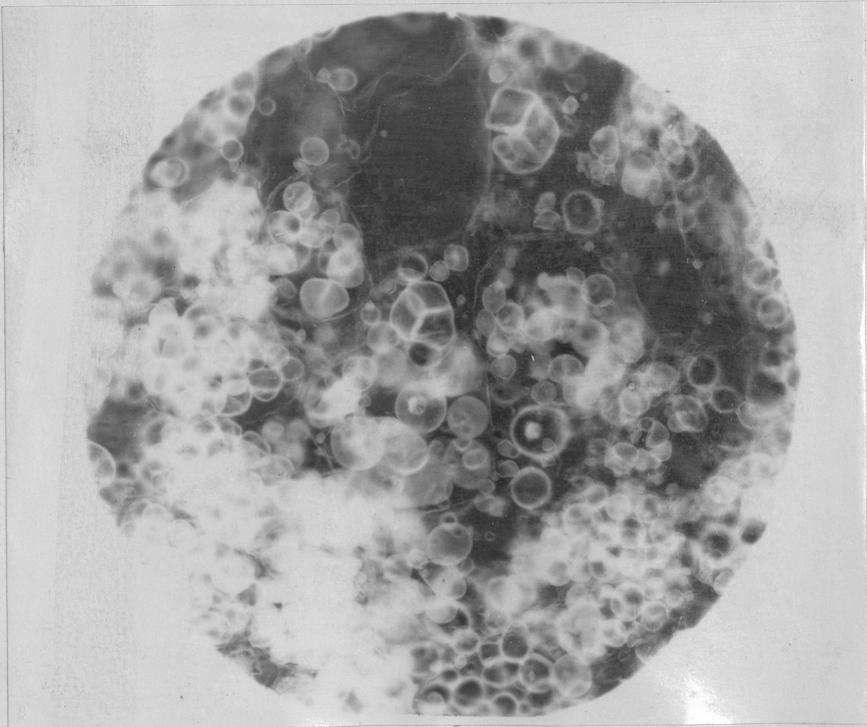
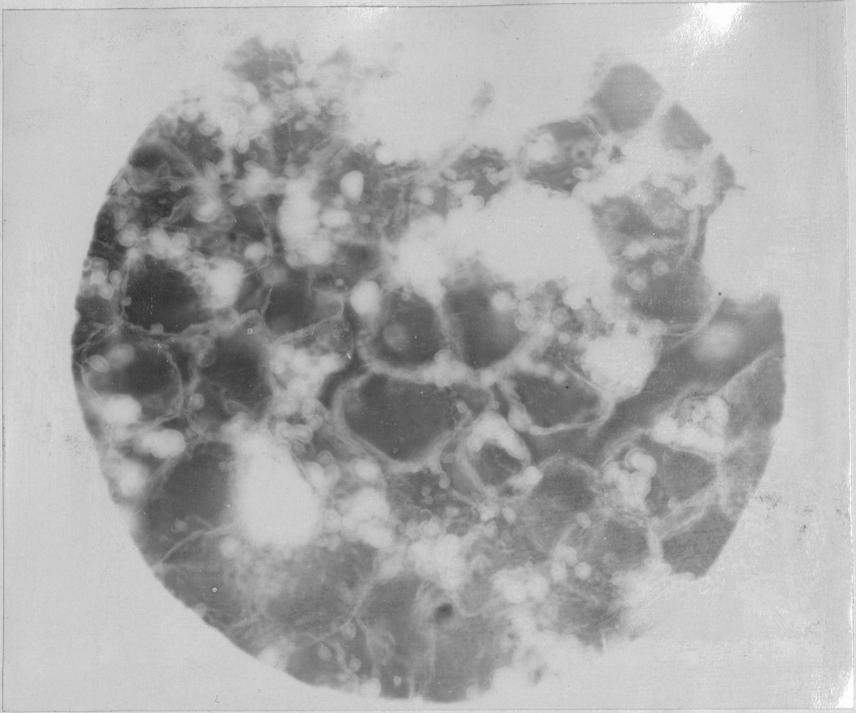


Plate 11. Starch grains of scorn stained
in iodine. x270.

Plate 12. Starch grains of sweet potato
stained in iodine. x270.

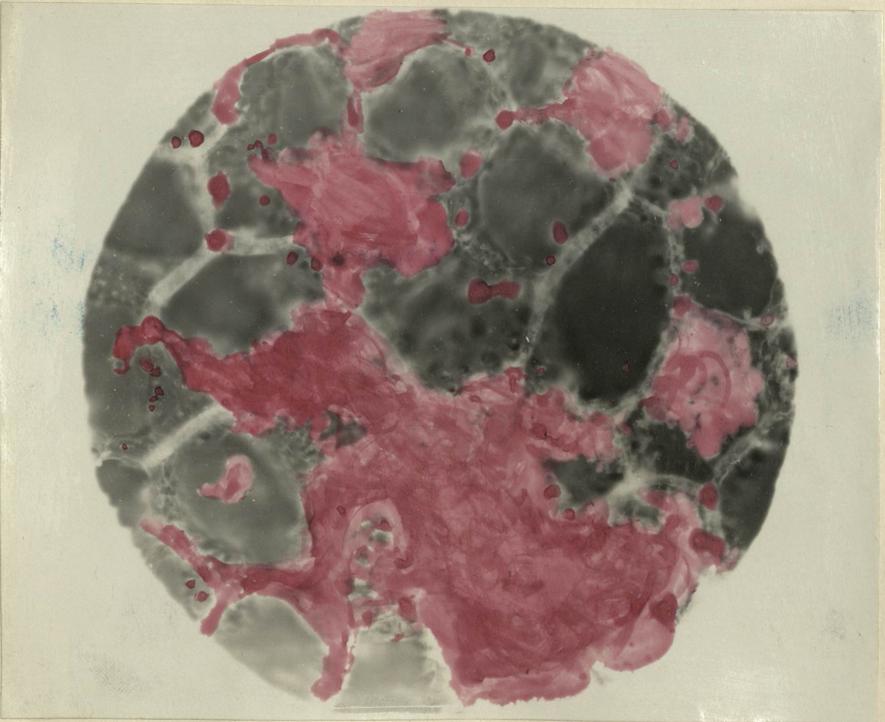
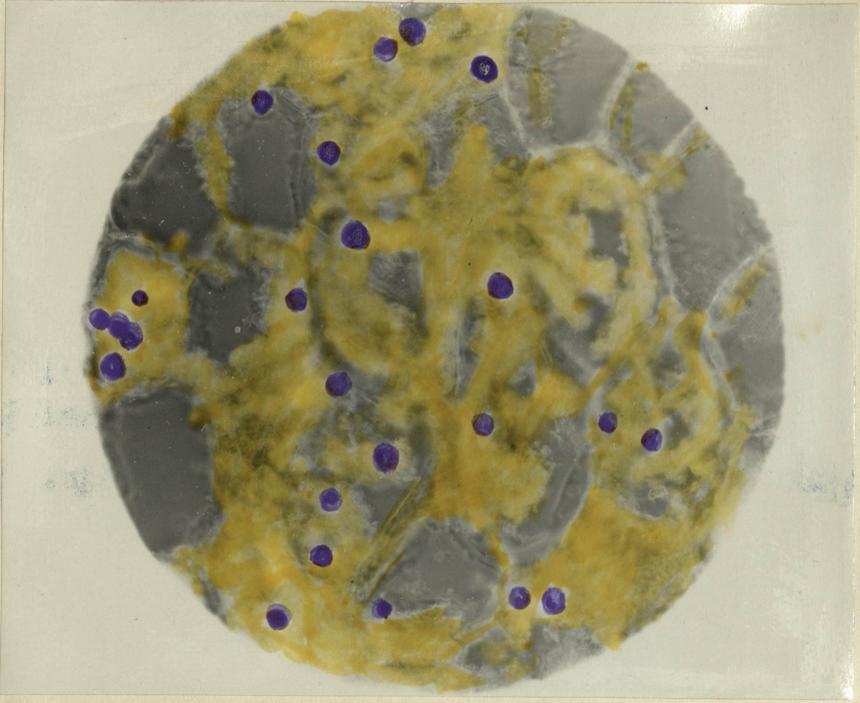


Plate 13. Section of peanut mounted in
castor oil-iodine, showing starch and protein. x270.

Plate 14. Section of peanut stained with
Sudan III showing masses of oil globules. x270.

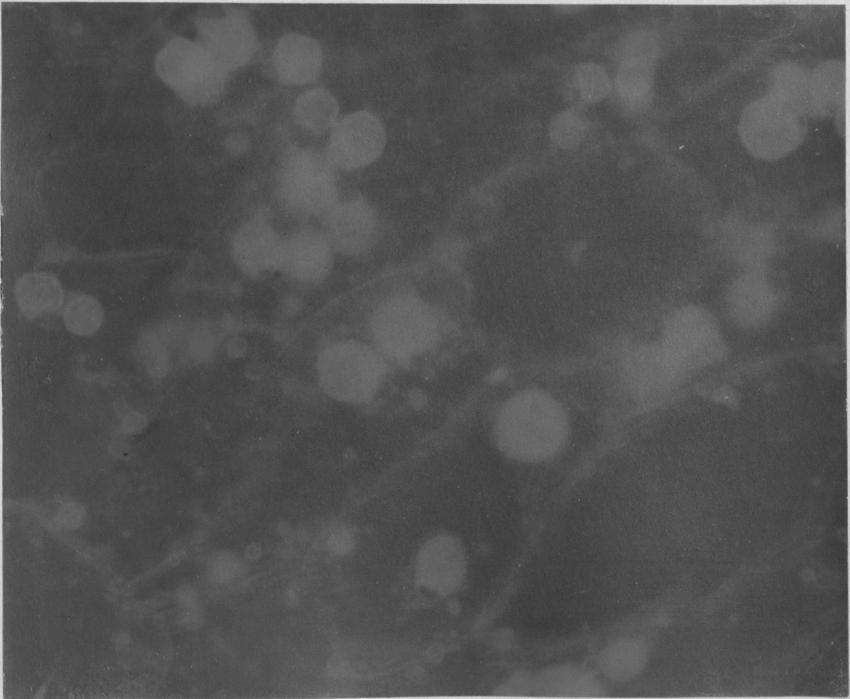
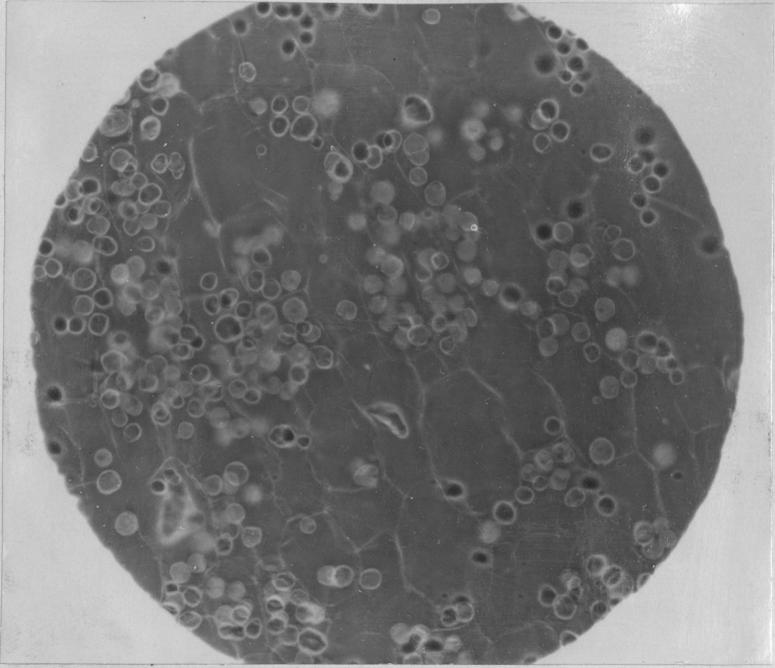


Plate 15. Aleurone grains in the cells
of castor bean. x270. Stained and mounted in cas-
tor oil-iodine.

Plate 16. Section of castor bean with
aleurone grains. x570. Exposure $\frac{1}{8}$ minute,
sensitive portrait paper.

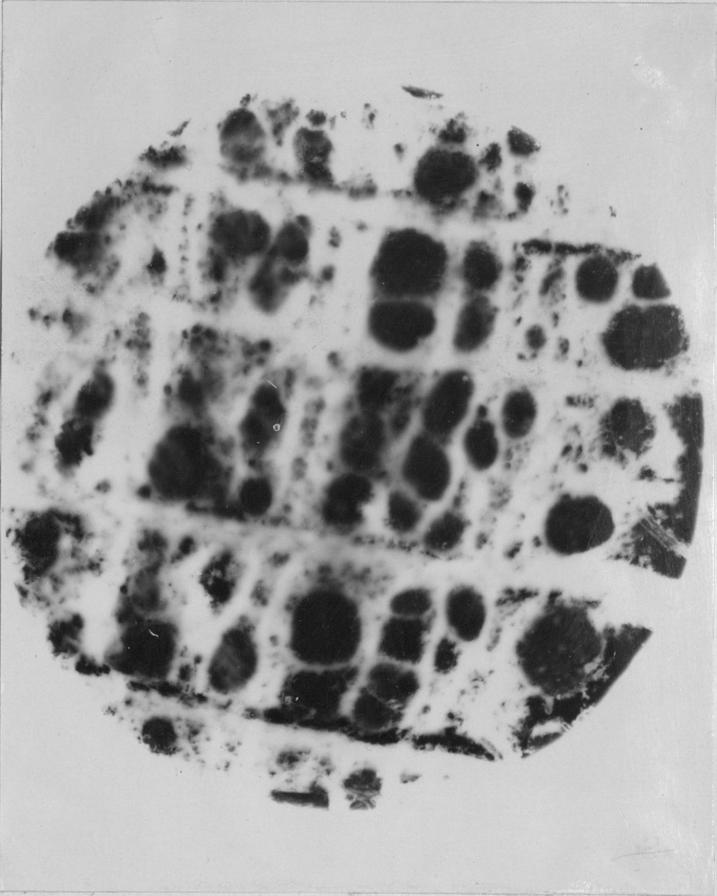


Plate 17. Section of *Ulmus fulva*,
Slippery Elm, showing masses of mucilage in the
cells of the phloem. x60. Stained in methylene
blue, mounted in dilute glycerine.

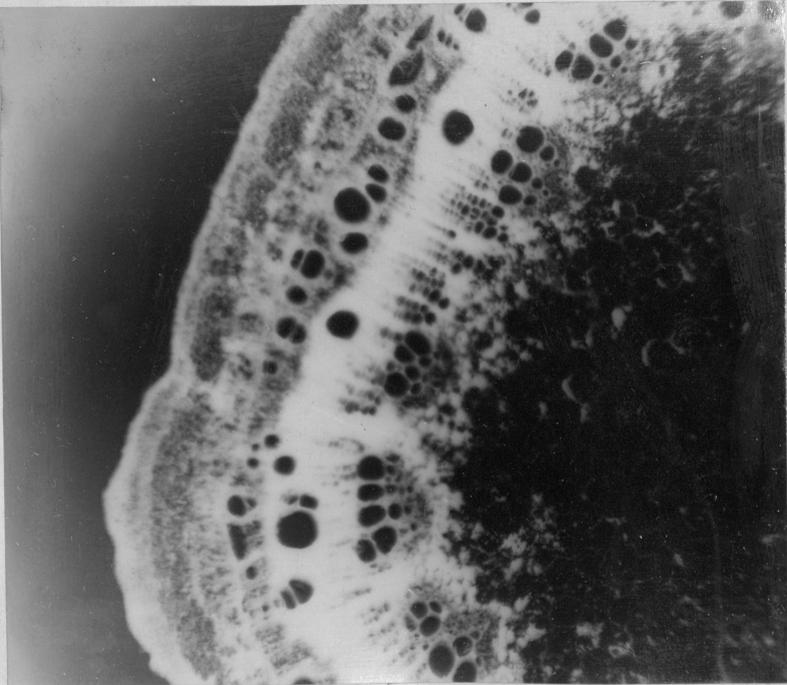


Plate 18. Section of Magnolia stem
stained and mounted in castor oil-iodine to show
reserve food. x60.

Plate 19. Section of Hops stem cut fresh
and mounted in castor oil-iodine to show reserve
food. x60.

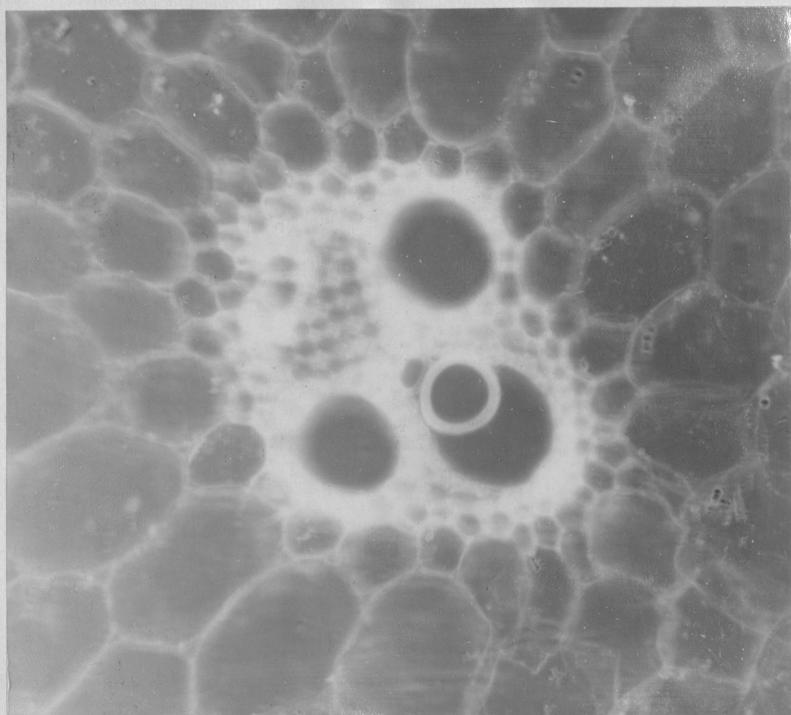
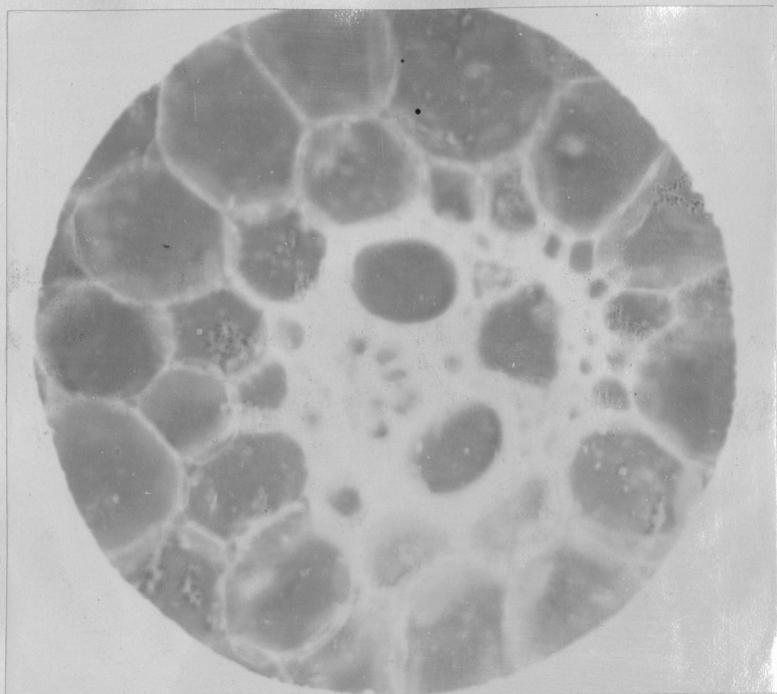


Plate 20. Vascular bundle of young cane.

x270. Section cut from the first internode above the permanent root system. Stained in safranin.

Plate 21. Vascular bundle from older cane

stem. x270. Section cut from the first internode above the permanent root system. Stained in safranin.

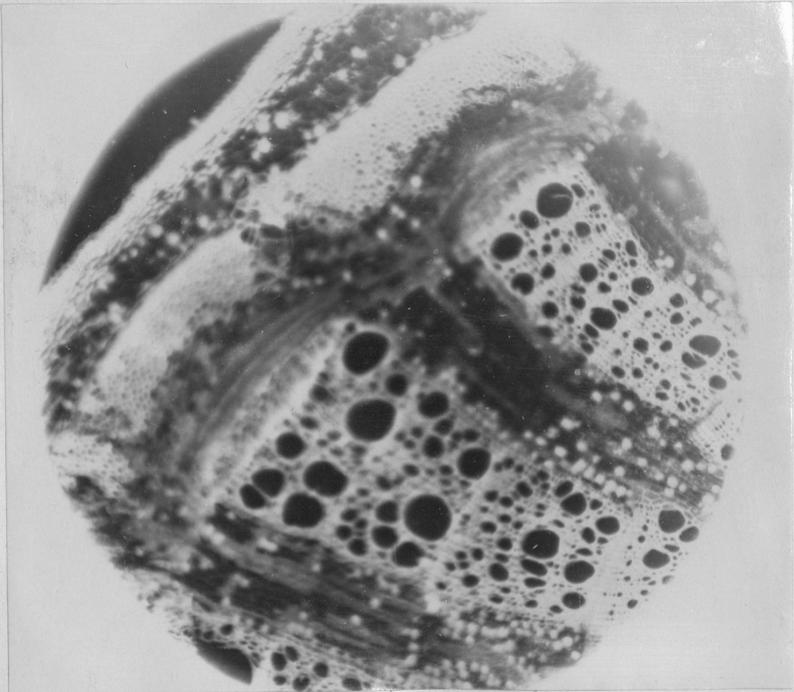
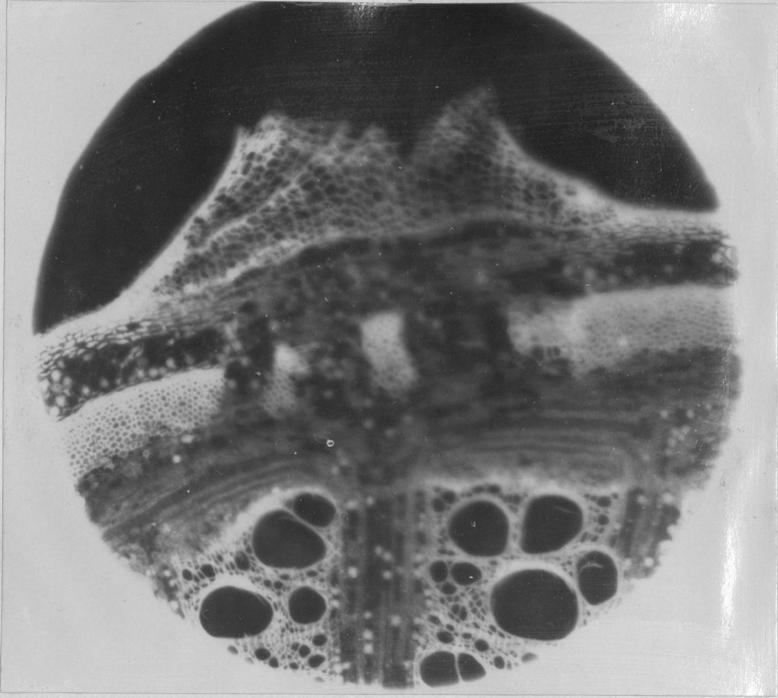


Plate 22. Lenticel on Aristolochia sipho stem. x60. Section cut at 35 mikrons, stained in Pianeze IIIb.

Plate 23. Four year old Aristolochia sipho stem. x60. Illustrating rings of growth.

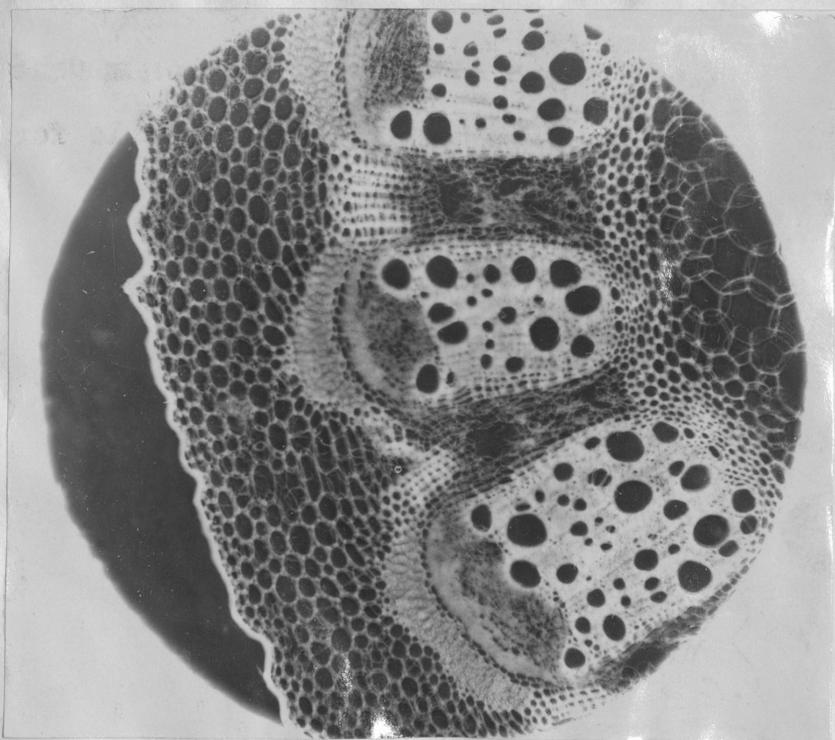
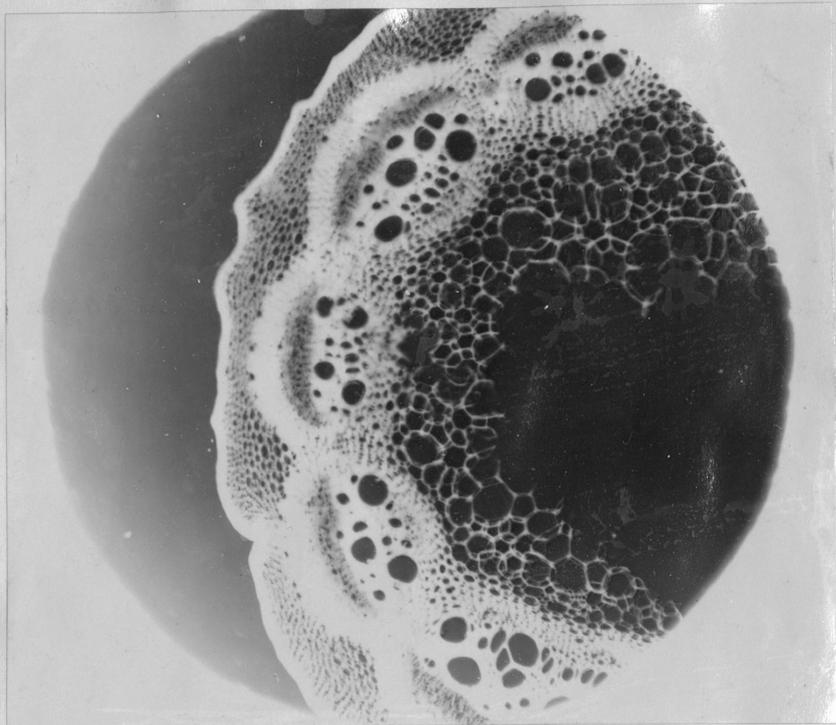


Plate 24. Young stem of *Menispermum Canadense*. x60. Cut fresh and mounted in chloral hydrate.

Plate 25. Rhizome of *Menispermum Canadense*. x60. A dicotyledonous stem suitable for a study of secondary thickening.

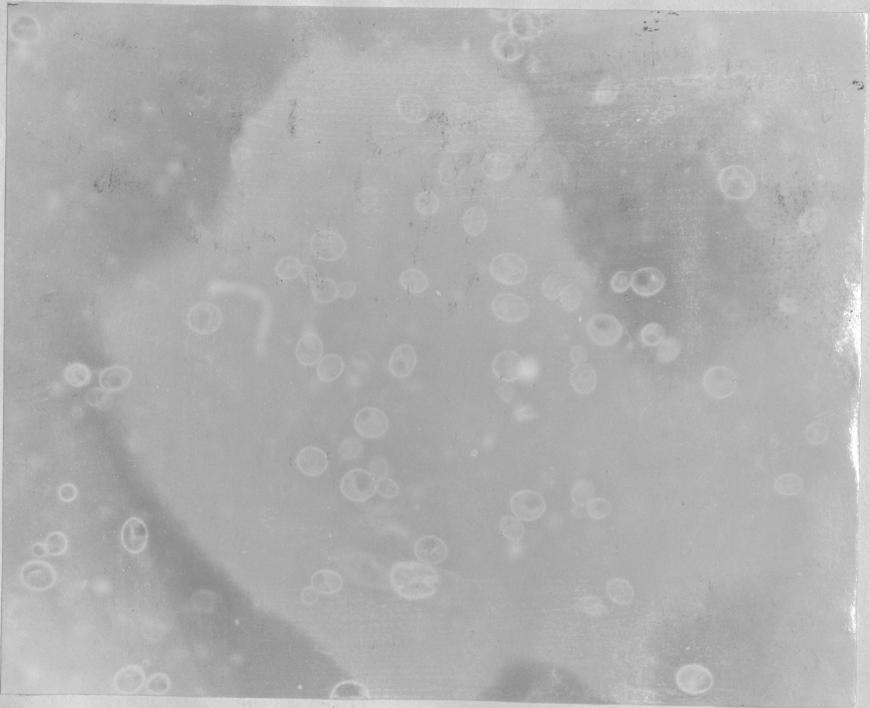
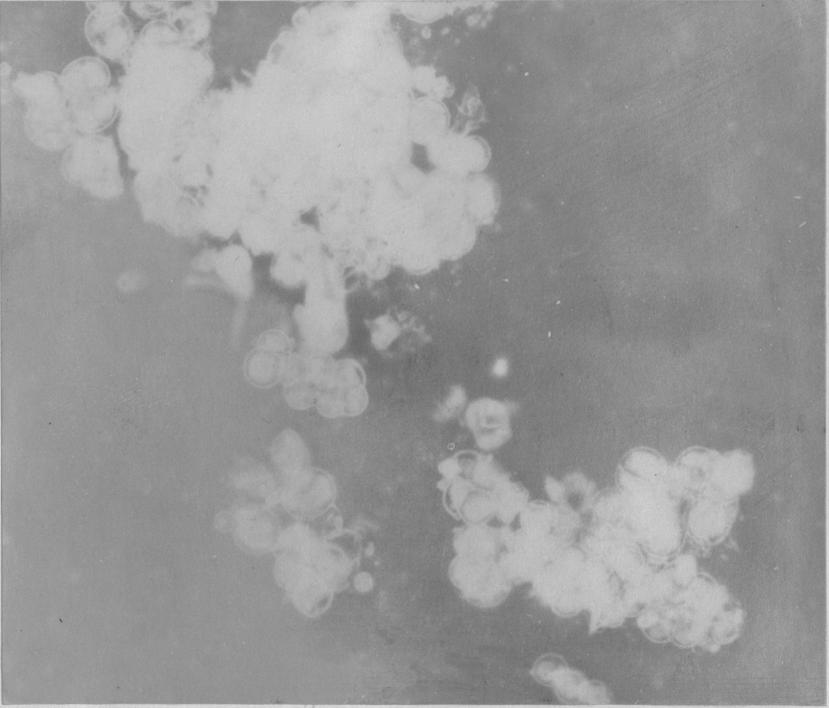


Plate 26. Pleurococcus. x570.

A mass of cells showing method of division.

Plate 27. Yeast budding. x570.

Exposure $\frac{1}{2}$ minute. Sensitive portrait paper.

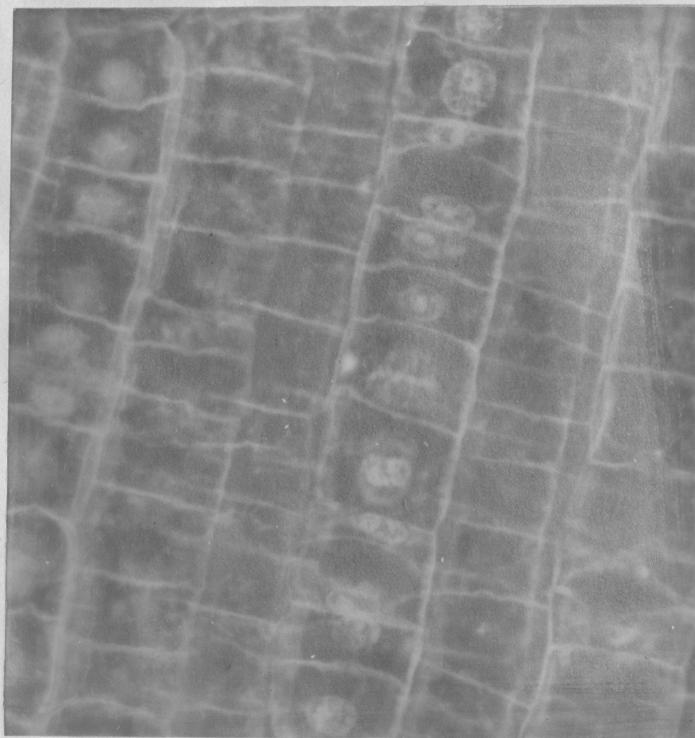
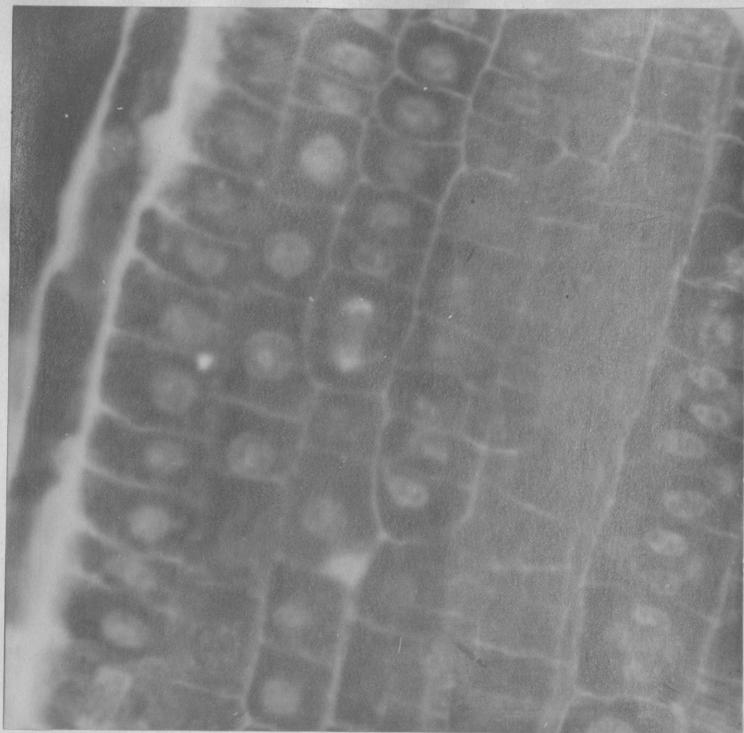


Plate 28. Dividing cells from the onion
root tip. x570.

Plate 29. Mitosis as illustrated in the
onion root tip. x570.