MANUAL OF PLANT HISTOLOGY.

THOMAS AND DUDLEY.

A

LABORATORY MANUAL

—OF—

PLANT HISTOLOGY

—BY—

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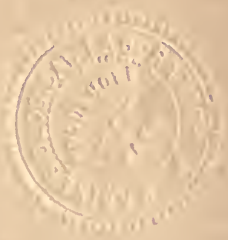

CRAWFORDSVILLE, INDIANA.
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The Journal Co.,
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ERRATA.

p. VIII, last line, for "Standford" read Stanford.

p. XV, line 37, add Roots of Dicots.

p. 45, line 2 from bottom, for "pillar" read arm.

p. 46, line 5, for "F" read N.

p. 54. line 13, for "Magnenta" read Magenta.

p. 54, line 20, for "c. m." read m. m.

p. 64, line 22, for "magnification" read size.

p. 65. line 3, for "1-1000" read 1000.

p. 69, line 16, for ' demonstraied" read demonstrated.

p. 70, line 20, after "p. 362" add 365-68.

p. 74, line 3, before "p. 118" insert p. 100.

p. 75, line 11 and 12, for "4, 5," read (a), (b).

p. 79, line 2, for "later" read latter.

p. 80, line 7, add p. 47-68.

p. 80, line 29, for "p. 11" read p. 61.

p. 90, line 2 from bottom, after "Fig" add p.

p. 91, line 12, add Fig. 27.

p. 95, line 24, for "vacular" read vascular.

p. 96, line 2 from bottom, for "reserviors" read reservoirs.

p. 97, line 21, for "p. 40" read p. 141.

p. 102, lines 15 and 25, for "vacular" read vascular.

p. 103, line 4, for "management" read arrangement.

p. 104, line 8, for "monocot" read Monocots.

p. 104, line 10, for "Cucurbit" read Cucurbita.

p. 108, line 1, for "System" read Systems.

INTRODUCTION.

Almost every thoughtful teacher of botany in the colleges and universities of our country is confronted by two problems in connection with his laboratory instruction. He is forced to provide a course which shall give the general student a fair knowledge of what the teacher deems the most important phases of plant life; and on the other hand, if a conscientious instructor, he will encourage students to advanced work, inaugurating courses which are intended not only to inform the mind, but to train the powers of observation, comparison and scientific judgment, and finally produce the investigator capable of pursuing problems of science without aid or admonition, if not without suggestions from his professor.

Some ten years ago, the present writer, then in charge of the Histology and Cryptogamic Botany at Cornell University, attempted a revision of his laboratory course in plant anatomy, in order to adapt it to the advanced courses, chiefly in Cryptogamic Botany, which followed. The result was a small hand-book, privately printed in 1886, entitled, "Anatomy and Histology of Plants," which evidenced the author's desire to impart some special knowledge of tissues as a foundation for more serious work in any subsequent subject involving the use of the microscope. It soon appeared, however, that better methods in the preparation of soft tissues and delicate organisms must be adopted, if any great advance was to be made toward the solution of problems of structural development.

It may here be mentioned that imbedding and cutting serial sections of delicate plant tissues, had not been put in practice in

American botanical laboratories previous to that time, and even in Germany it was but little in vogue. To study better methods of microscopic manipulation in this and other directions, was the object of the writer in spending the year 1887–'88 in the German laboratories, where he used for the first time the collodion method of imbedding. It was seen more and more clearly that in the future, students were to be trained to useful work in biological investigation chiefly through a mastery of microscopical technique, and a thorough knowledge of tissues and cell contents with their behavior under the influence of reagents. The changes in methods in the histological course brought about during the four years following 1888, were made to bear upon the work of students taking the courses on the higher and lower Cryptogams, with most excellent results. Such changes were included in the plans for a revised manual, carefully drawn up in 1892.

Mr. Mason B. Thomas, an undergraduate, then Fellow in Botany in the writer's laboratory, 1888–91, and afterward Profes- of Biology in Wabash College, was invited to assist in this work. During his university course he had been able to render me invaluable assistance, by refining and abridging the process of imbedding in collodion, and by devising various laboratory appliances connected with it (still remaining in the laboratory at Cornell), some of which are described in his papers published in 1891 to 93, * and detailed at some length in Atkinson's "Biology of Ferns" (1894), particularly in Part II., Chapter I.

The exactions of work since 1892, in an entirely new field have obliged me to abandon rewriting the Manual. At my request, Professor Thomas has done this, so far as it seemed necessary. He has also prepared the part on technique (Part I.), as well as plates, selecting the illustrations from his many beautiful preparations made while at Cornell University and since that time. The fact that some of the best laboratories in this country have adopted the methods formulated by him makes it particularly appropriate that he should write this part.

In it no attempt has been made at an exhaustive treatise but

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* (1) "The Collodion Method in Botany;" Rep. Am. Society of Microscopists, 1891; (2) "A Dehydrating Apparatus," Am. Monthly Microscopical Jol., Jan., 1891. (3) "Sectioning Fern Prothallia," The Microscope, Nov. 1893.

the matter is presented rather in the form of suggestions to those who may be at the beginning of their work in micro-chemistry or technique.

The tests for the different vegetable substances and the general properties of reagents have been taken from the best authorities on those subjects, and carefully tested.

I am responsible for the plan of the manual of directions (Part II.), for some of its phrasiology, and for the selections of most of the subjects used for study; and any imperfections in this part must be laid at my door. Nevertheless the plan has stood the test of many years thoughtful use in my own laboratory, and more recently in that of Wabash College; and Professor Thomas shares completely with the writer the belief that such an elementary course, most thoroughly taught, should be made the foundation for advanced instruction on the morphology of the higher and lower plants, and should enter into the education of a student for any independent work in anatomy, physiology, or biology.

If other teachers should find the work acceptable, we would remind them that a course of carefully prepared lectures should supplement the laboratory work, and we urge them to so present the subject, that the intergradations of tissues may not be overlooked, and the larger relations of great tissue masses and their beautiful adaptations to the necessities of the living plant, may be completely understood by the student. No true teacher will allow a student to consider these individual studies in an unrelated way. We have cited freely text books and reference works of unquestioned value, such as are to be found on the book-shelves of every good laboratory, but we have not made a practice of referring to original papers, as it would be for the most part out of place in a work of this kind. But this does not release the teacher from the duty of placing the most important papers bearing directly on a subject of study within the reach of the student and requiring him to look them over.

The inconvenience of using plates placed at the end of a book will not be great, and is offset by the fact that they are removed from the unavoidable scrutiny of the student as he is executing his own drawings, but any defect in this or any other direction noticed and communicated by a teacher may be rectified in another edition.

To the student we would say that they, as men fitting themselves for professional or semi-professional scientific careers, have certain duties to themselves, entirely independent of the formal requirements of the instructor. Their aim should be a complete familiarity with the methods suggested, a comprehensive and scientific knowledge of as many facts as possible, and an ability not only to execute but to finally plan their own work, and themselves solve their scientific problems. To this end they should not, even in this elementary course, content themselves with the lines laid down, but should consult all books suggested in the studies given in the manual, and read carefully the passages to which reference is made. They should miss no opportunity to learn of a new work or an original paper in botany, or any fact concerning the mode of work of any genuine contributor to the literature of the science. We have an especial sympathy with the ambitious student whose superior training or skill enables him to accomplish more than the average students. For him are suggested the additional studies in the hand-book, and he will always find his instructor ready to advise him in regard to further reading. Both teacher and pupil should recognize the fact that in the present day, a sure foundation may be laid in undergraduate years, for a subsequent successful professional career, if the pupil thoroughly learns the use of his tools and pursues his chosen science with the zeal that belongs to his time of life.

It is with genuine regret that I lay down this work as well as the particular plans which were the motive of it, for broadening and deepening the training of American botanical students; but in doing so, I am sure that in the hands of Professor Thomas it will arrive at a better development than in my own, and that his efforts in this field will find nothing but appreciation.

The authors wish to express their indebtedness to Mr. E. W. Olive, Instructor in Biology in Wabash College, for the many ways in which his services have lightened the labors in the preparation of this manual.

WILLIAM RUSSEL DUDLEY,

August, 1894. Leland Standford, Jr., University.

WORKS OF REFERENCE.

In the selection of the list of books and periodicals below, it has been the intention to give only the more general ones and those that should be in every botanical laboratory. The list is in no sense intended to be a complete one, and it is expected that the student will have at his disposal, a number at least, from each of the groups.

For special or advanced work the original papers and monographs, on each particular subject considered, must be obtained.

General Botanical Works.

Bastin, College Botany; Engelhard & Co., Chicago, 1890.
Bennett & Murray, Cryptogamic Botany; Longmans & Co., London, 1889.
Bessey, Botany for High Schools and Colleges; Holt & Co., N. Y., 1892.
Campbell, Structural and Systematic Botany; Ginn & Co., Boston, 1890.
DeBary, Comparative Anatomy of Phanerogams and Ferns; Oxford Press, London, 1894.
Engler and Prantl, Die Naturlichen Pflanzen Familien; Englemann, Leipzig; issued in parts and not yet complete.
Frank, Lehrbuch der Botanik; Engelmann, Leipzig, 1893.
Goebel, Outlines of Classification and Special Morphology; Oxford Press, London, 1887.
Gray, Structural Botany; Am. Book Co., New York. 1879.
Sachs, Gesammelte Abhandlungen ueber Pflanzenphysiologie; Engelmann, Leipzig, 1893.
Sachs, The Physiology of Plants; Oxford Press, London, 1887.
Sachs, History of Botany; Oxford Press, London, 1890.
Vines, Physiology of Plants; Cambridge Press, London, 1886.
Vines' Text Book of Botany; Swan, Sonnenschein & Co., London, 1894.

Laboratory Manuals.

Arthur, Barnes, and Coulter, Plant Dissection; Holt & Co.. N. Y., 1887.
Bower, Practical Botany; MacMillan & Co., N. Y., 1891.
Davis, Text Book of Biology; Chas. Griffin & Co., London, 1893.
Dodge, Elementary Biology; Harper & Brothers, N. Y., 1894.
Dudley, Histology of Plants; Ithaca, N. Y., 1886.
Goodale, Structural Botany; Am. Book Co., N. Y., 1885.
Huxley & Martin, Practical Biology; MacMillan & Co., N. Y., 1889.
Parker, Elementary Biology; MacMillan & Co., N. Y., 1891.
Sedgwick and Wilson, Biology; Holt & Co., N. Y., 1889.
Spalding, Introduction to Botany; Heath & Co., Boston, 1893.
Strasburger, Practical Botany; Swan, Sonnenschein & Co., London, 1893.

Structural and Technique.

Atkinson, Biology of Ferns; MacMillan & Co., N. Y., 1894.
Methods for treatment of tissues; structure.
Bausch, Manipulation of the Microscope; Rochester, N. Y.
Manipulation and care of instrument.
Beale, How to Work With the Microscope; London, 1880.
Structure and methods.
Beherens, Guide to the Microscope in Botany; Boston, 1885.
Carpenter, The Microscope and its Revelations; Philadelphia, 1891.
Manipulation and care of instruments; also structure.
Clark, Practical Methods in Microscopy; Heath & Co., Boston, 1893.
Fry, The Microscope and Microscopical Technology; New York, 1892.
Structure, manipulation and methods.
Gage, The Microscope and Histology; Ithaca, N. Y., 1894.
Care and manipulation of instruments; also methods of mounting.
Goodale, Physiological Botany; Am. Book Co.
Lee, Microtomists Vade Mecum; Philadelphia, 1890.
Methods.
Strasburger, Practical Botany.
Van Heurck, The Microscope, Construction and Management; D. Van Nostrand Co., N. Y., 1893.

Botanical Micro-Chemistry.

Poulsen, Micro-Chemistry, Trans. by Trelease; Ginn & Co., Boston, 1886.
Zimmermann, Botanical Micro-Technique, Trans, by Humphrey; Holt & Co., N. Y., 1893.

Botanical Journals, and Periodical Publications.

Annals of Botany; Oxford, Clarendon Press, London.
Morphological, Systematic, and Physiological.
Am. Monthly Microscopical Jol.; Washington, D. C.
Microscopical methods and histology.
Botanical Gazette; Lake Forest, Ill.
Morophological, Physiological, and Systematic.
Botanisches Centralblatt, Cassel; contains original work together with Bibliography of Current Botanical Literature, 1880——
Bulletin of Torrey Botanical Club, N. Y.; largely systematic.
Just's Botanischer Jahresbericht, Berlin; Bibliography of Botanical Literature, 1873——.
Annales des Sciences Naturelles (Botanique); Red. par A. Brongniart et J. Decaisne, Paris, 1854——; chiefly original papers.
Botanische Zeitung, Leipzig; original papers and Bibliography, 1843——

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Laboratory Manual of Plant Histology.

Part First.

MICRO-CHEMISTRY AND TECHNIQUE.

REAGENTS.

Certain stains and reagents are absolutely indispensable for laboratory work while others although not indispensable are nevertheless very desirable and become necessary in thorough and advanced investigations. The following is an alphabetical list of the more important chemicals, reagents, and stains, together with tests for the more frequently occurring vegetable substances.

Alcohol.

For all ordinary laboratory manipulations commercial 95 per cent. Ethyl alcohol is sufficiently strong, and it is only when all trace of water is to be removed from a specimen, that alcohol of absolute strength is needed. Absolute alcohol has like osmic acid the property of rendering protoplasm rigid and can therefore be employed to advantage in studying the more intricate structures of protoplasmic bodies; as for example, nucleus or cell division. Common alcohol readily removes air from intercellular spaces, especially if heat is applied. It is also extensively used in hardening tissues, different strengths being employed to prevent its strong avidity for water causing too great a shrinkage of the protoplasm from the cell wall. Tissue hardened in this way can be softened again by soaking it in water. Alcohol is used for dehydrating sections that are to be mounted in balsam and for dissolving many fats, resins, and oils from plant tissues.

It is a solvent for chlorophyll, and is used in the preparation of many stains. If tissue containing inulin is kept in alcohol, the inulin is precipitated within the cell in the form of sphaero-crystals. Many sphaero-crystal forming substances separate in the same way, e. g., hesperidin.

Acetic Acid.

Glacial acetic acid is a valuable clearing agent. In 2 per cent. solutions it is good for clearing up the cell contents and in studying the nucleus or protoplasmic structure. In strong solutions it dissolves the cell contents and makes the cell wall clear. It is also used in testing for oxalate crystals which are insoluble in it, but which dissolve without effervescence in HCl, while carbonate crystals dissolve with effervescence in both.

Alum.

Alum is employed as a mordant in various staining processes, as, for example, in Frey's haematoxylin. It is also used to render more visible cells that have become too transparent by treating with KOH.

Ammonia.

Strong aqueous ammonia is sometimes used in preference to KOH where the action of the latter would be too violent. If tissue with thick walled cells be placed in nitric acid and then in ammonia, the middle lamella of the cells will be colored yellow. Ammonia is also used in the preparation of certain stains and in Schweizer's reagent for dissolving cellulose without essentially changing its composition.

Anilin Chloride.

Anilin chloride is used as a test for lignin, one of the constituents of wood. The sections to be treated are placed in a dilute solution until they are thoroughly saturated. They then assume a pale yellow color which is much deepened upon the addition of HCl. This is Hoehnel's test for lignin.

Argentic Nitrate.

A dilute alkaline solution of silver nitrate when fresh is used as a test for living protoplasm. The aldehyde which is contained in the living protoplasm precipitates metallic silver free in the solution and colors the protoplasm dark. Dead protoplasm is not affected in any way.

Chloroform.

Chloroform is chiefly used as a solvent for fats, resins, and oils; also to some extent in the preparation of chloroform balsam. According to VanWisselingh it is a solvent for the various suberin constituents.

Calcic Chloride.

This salt in an aqueous solution is used as a mounting fluid and not infrequently it is found useful for clearing. The tissue to be cleared is moistened with a little water and some of the powdered salt is then sprinkled on it. It is heated gently until most of the water has been evaporated. The whole is again moistened and mounted in glycerin when it becomes clear.

Cupric Sulphate.

The pure salt in an aqueous solution is largely used in the detection of sugars. The test employed by Trommer is as follows: The tissue under examination is allowed to remain in a concentrated solution of the salt for about ten minutes when it is rinsed with distilled water and placed in a boiling mixture of water and and potassic hydrate. The reaction with cane sugar in the section is to turn the cells containing it a light blue, while with grape sugar (glucose), the reaction causes the cells to become clouded by the deposition of a fine flocculent or granulated orange precipitate of reduced oxide of copper. Dextrine when not mixed with protein compounds assumes a vermilion color. Protein compounds in young cells, with the above tests, turn a violet color. We are thus enabled to detect the presence of two and often three kinds of sugars in this reaction.

Carbon Disulphide.

This agent is used chiefly as a solvent for fats, oils, wax, etc., also for carotin, a coloring matter with the same composition as xanthophyll, chlorophyll yellow, etc.

Carbolic Acid (Phenol).

This acid is sometimes used as a solvent for fat and fatty oils. When mixed with three parts of turpentine, it makes a good clear-

ing agent for most plant tissues. With carbolic and hydrochloric acids, lignified cells become yellowish green. The test is best made by adding a few drops of concentrated HCl to some crystals of carbolic acid, warming slightly, and when cold, add HCl enough to dissolve any crystals that may have separated out. This gives a solution of crystals in just enough HCl to dissolve them, and in this the tissue is placed. (See Zimmermann's Microtechnique, p. 145.)

Chromic Acid.

Chromic acid in strong solutions dissolves the cell wall rapidly except in those cases where it may be cutinized, silicified, or corky. If the action is allowed to continue, the cutinized wall will finally dissolve. In dilute solutions the acid causes the cell wall to swell and often brings out very clearly the markings or stratifications, as in those of starch grains. Chromic acid is sometimes used in dilute solutions as a hardening agent.

Cuprammonia.

This is the so-called Schweizer's reagent and is effective only in fresh solutions. It is prepared by adding to an aqueous solution of copper sulphate some sodium hydrate, until a precipitate of copper hydrate is formed. The precipitate is filtered out and washed with hot water, after which it is dissolved in as little ammonia as will take it up. It forms a deep blue solution and will dissolve quickly cotton fibers. Cell walls of pure cellulose swell and are readily dissolved by the solution, but, if they contain lignin or suberin, the reagent will not act until these substances are removed in some way, e. g., by Schulze's maceration method.

Cleaning Mixture.

A cleaning mixture that works rapidly and removes balsam at once from the slide is made by adding two parts of strong HNO_3 to three parts of concentrated H_2SO_4. The mixture must be kept covered as the fumes are very disagreeable. This mixture cleans glass very quickly and does not injure it. A dichromate cleaning mixture is made by dissolving 200 grams of potassic dichromate in 1000 c. c. of water and then adding to the mixture 1000

c. c. of H_2SO_4. Much heat is generated by the action of the acid and the operation should be performed in a beaker or earthen dish, since the heat would probably crack a bottle should an attempt be made to make the mixture in one. This cleaning mixture will after a time remove balsam and sealing agents from glass, but it is not so rapid in its action as the nitro-sulphuric acid mixture, nor is it so disagreeable to handle, and may therefore be better adapted for the use of the general student.

Collodion.

This is best prepared by dissolving pure gun cotton (pyroxylin) in a mixture of equal parts of pure ether and 95 per cent. alcohol. A 2 per cent. and 5 per cent. solution will be needed. These can be made by dissolving 2 and 5 grams respectively of gun cotton in mixtures of 100 c. c., equal parts of pure ether and alcohol. The solutions should be kept in tightly stoppered bottles to prevent evaporation of ether and consequent thickening of the collodion. More satisfactory results will be secured by using gun cotton in the preparation of collodion than by employing ordinary commercial collodion or celloidin.

Ether.

Ether is used with equal parts of alcohol in the preparation of collodion. Ether vapor is useful for sealing collodion sections to the slide and is often a valuable agent as a solvent for oils, fats, and resins.

Glycerin.

Glycerin is quite an important substance in microscopic manipulation. It is used largely as a mounting medium and preservative. It evaporates slowly but absorbs water readily from the air. Mounts in it should therefore be sealed with a water tight cement shortly after preparation. Sections mounted in glycerin, unless stained with a very permanent stain, are liable to become transparent and of but little use. Glycerin with gelatin is used to make glycerin-jelly, a very convenient mounting medium for a large variety of plant tissues. The tissue to be preserved in the jelly can be mounted directly without dehydrating in alcohol, but in most cases it should be first hardened to prevent shrinking.

If tissue containing inulin is placed in glycerin, the inulin separates out in the form of sphaero-crystals. Kraus has used the same agent as a test for sugar. Iodine glycerin is used largely as a medium for the study of protein granules. The reagent is made by dissolving a little iodine in some glycerin in which has been placed a little iodide of potassium. Glycerin is often used as a clearing fluid and is especially good as applied to Hanstein & Russow's methods. (See Poulsen, Botanical Micro-Chemistry.)

Hydrochloric Acid.

This is a valuable macerating agent for woody tissues. It is also used to distinguish between oxalate and carbonate salts. The former when treated with the acid dissolve without, and the latter with effervescence. Pringsheim has used the acid as a test for hypochlorin, a compound of chlorophyll bodies. The tissue to be treated is sectioned and placed directly in the acid, where it is allowed to remain 2 or 3 hours. The hypochlorin will separate out in the form of brown spherical masses which later become needle-shaped crystals. Hydrochloric is also used in connection with nitric acid as a cleaning mixture.

Iodine.

Iodine is one of the most useful agents in micro-chemistry. It is used in solutions of glycerin, alcohol, or an aqueous solution of iodide of potassium, the latter being the one usually employed in most tests. Dilute solutions generally give the best results and the reaction is not obscured by the intense color of the reagent. One quite well adapted for most tests is made by dissolving 1 gram of iodine and 5 grams of potassic iodide in 100 c. c. of water, yet even for some purposes, a solution of one-half this strength is desirable. Iodine is sparingly soluble in water and in cases where the effect of the pure agent is to be observed, it is better to put a little metallic iodine in water under the cover glass at the side of the preparation. In a solution of zinc chloride, iodine forms the so called Schulze's reagent, which is very generally used as a test for cellulose. Iodine is an infallible test for starch, coloring it in the presence of water a rich blue. If the reagent is too strong, the

starch will be colored a dark brown, and in the presence of an absolute alcoholic solution of iodine, the same color is secured, but if water is present, the color will be blue. This forms a ready test for the presence of water in alcoholic solutions. Cellulose membranes are colored by iodine a pale yellow or deep brown. If a mixture of two parts of sulphuric acid and one of water be added just before or after the test, the reaction will give a blue color, while lignified cells if present will turn brown.

Iodine kills protoplasm quickly coloring it a deep brown. Alcoholic solutions of iodine deteriorate by standing, due to the formation in them of hydriodic acid. The deterioration is greatly augmented by the action of light and the solution should therefore be kept in the dark. Two solutions of different strengths should be in the laboratory, since it is important that the correct strength be employed in the different tests.

Millon's Reagent.

This reagent is used for the detection of albuminoid substances, it readily causing them to turn a strong red color. The reagent is prepared by pouring over some pure mercury an equal quantity by weight of strong HNO_3. If the solution is not complete, heat the mixture, then pour over it twice its volume of water. After allowing it to stand a few hours, decant the clear portion for use. The reagent will act only when used in fresh solutions.

Nitric Acid.

Nitric acid is used with potassic chlorate as a macerating agent. When added alone to tissues, it causes the protein matters to turn a bright yellow. The reaction is made more apparent upon the addition of ammonia. According to Hoehnel, the acid forms a good test for suberin. It is also used for clearing tissue of starch, causing the grains to swell and soon dissolving them.

Oxalic Acid.

An alcoholic solution of the acid is very useful in bleaching sections that have previously been too deeply stained. Dilute aqueous solutions are employed with some stains for various tissues, and a concentrated solution dissolves pectose after treatment with potash.

Perosmic Acid.

This acid is very volatile and has an extremely disagreeable and poisonous odor; it therefore must be kept in a sealed glass tube. It is usually employed in an aqueous solution of 1 per cent. strength, and this should be kept tightly corked in a dark place. The acid is most useful for killing and fixing at once living protoplasm. It is, therefore, very helpful in studying nuclear and cell division, since it prevents immediately all further change.

Oils and fats are discolored by the acid, due to its reduction and the deposition of metallic osmium. As a hardening agent it is used with 9 parts of 25 per cent. chromic acid, and it not only hardens, but stains simultaneously meristematic tissue.

Potassic Dichromate.

Potassic dichromate is often used as a substitute for chromic acid in hardening, and seems to give about the same results. It is used especially for hardening resin masses and sometimes for the detection of tannin. After continued action it colors cells containing the latter substance a reddish brown.

Potassic Chlorate.

This salt with HNO_3 forms Schulze's macerating agent, which is especially useful in destroying the middle lamella and for the isolation of wood cells. The macerating is aided by the application of heat. Since the fumes arising from the mixture readily corrode metal, it is very important that the operations with the agent be performed in a room that does not contain any delicate instruments. Schulze's agent is also used in the detection of suberin. The suberized cell-walls resist for a long time the action of the mixture but finally break down, and a part of the suberin forms ceric acid, which is readily soluble in potassic hydrate, ether, chloroform, etc.

Paraffine.

Hard and soft paraffine are both useful; the former with a melting point of about 45°C., the latter with 33°C. Different melting points can be secured by mixing in different proportions

the hard and soft kinds. The melting point can be lowered by the addition of chloroform. It must be remembered, however, that before any attempt is made to section imbedded tissue, *all* of the chloroform must be driven off in the infiltrating oven, otherwise the paraffine will be too soft for support. Turpentine can be used in place of the chloroform and is perhaps quite as good.

Phosphoric Acid.

Phosphoric acid is sometimes used to remove water from tissues, and when added to any containing crystalloids, it causes the latter to swell.

Rosalic Acid.

This acid is used in connection with sodic carbonate as a test for vegetable jelly, staining it red. It is useful in coloring the callosities of sieve-tubes and in bringing out the general structure of cribrose tissue.

Sugar.

If tissue containing protoplasm is left for some time in a thick syrup of cane sugar and then transferred to $H_2 SO_4$, it will turn a red color. The reaction is not always a certain one. A 10 per cent. solution of sugar is very useful for pollen and for spore cultures.

Sulphuric Acid.

Sulphuric acid is used in connection with many tests and is also valuable in breaking down cellulose walls, without shrinking the protoplasm. This makes it especially important in demonstrating the continuity of protoplasm. It is also used in connection with iodine to determine the purity of the cellulose that makes up the cell walls of any tissue. In this test the tissue is first treated with a tincture of iodine, and sulphuric acid is then added. The walls will turn blue if they are composed of pure cellulose. Dilute sulphuric acid causes starch grains to swell, while with the concentrated acid they are dissolved. Pure cellulose walls are likewise dissolved by the acid, while cutinized ones resist its action. Fat bodies are not soluble in it, but they form small refrative drops.

Chlor-iodide of Zinc.

This is known as Schulze's reagent and is very useful in detecting the presence of cellulose. This reagent is made by pouring over metallic zinc some hydrochloric acid and then evaporating the solution with an excess of zinc present, until it becomes of a thin syrupy consistency. Add as much iodide of potassium as will be taken up, and then iodine until a saturated solution is obtained. Keep the reagent in the dark to prevent the formation in it of hydriodic acid.

Pure cellulose gives with this reagent a blue or violet color, due to the staining by the iodine of the amyloid which is formed by the action of the agent on cellulose. Wood, cork, and cutinized walls are colored yellow, while starch colors blue, but the grains soon become disorganized. Cells containing tannin are colored by the reagent red or violet. Fungus cellulose, unlike ordinary cellulose, remains uncolored by the action of this agent.

HARDENING AGENTS.

The subject of hardening agents is one of very great importance and presents a field in which much yet remains to be learned. The object of hardening is to bring the tissue in a condition to be either sectioned directly without crushing, or to allow it to be infiltrated with some substance that will hold it firm for cutting. The difficulty to overcome is to find an agent that will harden the tissue without shrinking it. The method employed to overcome this is to bring the tissue in contact with the dilute hardening agent, and then gradually increase its strength to prevent a violent action between it and the tissue. The former must always be present in a large excess in order that an equilibrium may not be established too quickly.

The exact strength of the hardening agent in which the tissue should first be placed is a matter of some uncertainty and can only be determined after experimenting with each sort of tissue. After the object is hardened, it should not be left any great length of time in the full strength of the hardening agent as it is liable to become brittle.

In arranging the tissue to be hardened it should be carefully trimmed and only the portions that are needed for examination placed in the agent. In the case of large pieces, as, for example, closed pistils, cut the parts open to allow the hardening agent to penetrate all parts of the object, otherwise, deterioration will result.

Alcohol.

This is one of the most frequently employed hardening agents, and for many plant tissues is all that could be desired. For most soft material 40 per cent. alcohol is dilute enough to begin the

hardening. From this strength it is transferred to 50 per cent., 65 per cent., 75 per cent., 85 per cent. and 95 per cent. alcohol respectively, allowing it to remain in each solution for about 24 hours, varying the time according to the nature of the tissue. If it is desirable to preserve the tissue for future examination, the hardening should cease with the 75 per cent. strength, and in this it should be kept until needed, when the hardening may be completed with the 85 per cent. and 95 per cent. strengths.

A very convenient apparatus for hardening plant tissues was invented by Schulze, and a modified form of it is recommended for laboratory purposes. It can be made as follows:

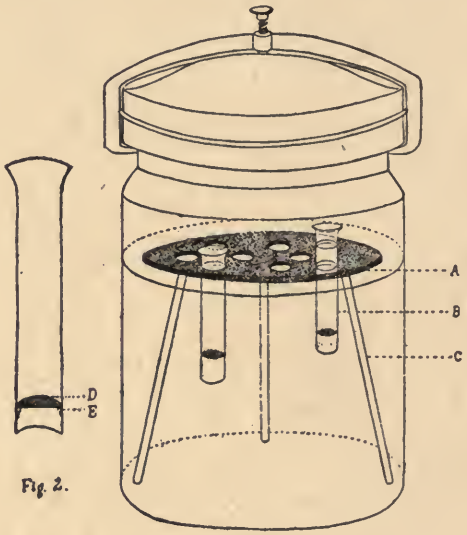

Fig. 2.

Fig. 1.

Fig. 1. Dehydrating apparatus.

Fig. 1. Apparatus complete, showing dehydrating tube B in place. A. plaster of paris diaphragm. C. Glass rod supporting the disk.

Fig. 2. Dehydrating tube. D. Chamois skin diaphragm. E. Spring holding the diaphragm in place.

In a 9x9 Whitall-Tatum museum jar a disk of plaster of paris is supported about 5 c. m. from the top by means of legs made of glass rods. The disk is perforated to allow tubes of various sizes, from 2–4 c. m. in diameter to pass through. These are the so-

called dehydrating tubes. The plaster of paris diaphragm can be made by first constructing a mould of the desired size, with a paper bottom and a card-board hoop for the outside. This must be placed on a level surface. The plaster of paris is then mixed with water and poured into the mould to about the depth of 1½ c. m. While it is yet soft the three legs can be inserted near the edge and holes for the dehydrating tubes cut in the disk with a knife or pressed out with glass tubing of convenient size. When the plaster is dry the hoop can be removed and the disk placed in position in the jar, which is then filled with alcohol to within about 2 c. m. of the under side of the plaster. The dehydrating tubes should be about 12 c. m. long and can be made by cutting off the bottom of large test tubes. In one end is placed a diaphragm of chamois skin, which can be fastened in position by means of a spring made of steel wire or ribbon and forced with the chamois skin in the tube. A rubber band around the tubes prevents them from falling through the holes in the disk and enables them to be lowered to any desired depth in the alcohol.

The tissue to be dehydrated is packed closely in the dehyrating tube and just enough 50 per cent. alcohol added to cover it. This is then quickly lowered through the hole in the disk, until the two liquids are at a level. After from 12 to 24 hours, by osmosis, the two liquids will be of the same strength. The tissue can then be taken out and placed in the infiltrating bath at once. This method of hardening has been tried on nearly all kinds of plant tissue, and in almost every case it was found to be successful. For the most delicate tissues where slow hardening is desired, 5 per cent. alcohol can be placed in the dehydrating tube, and thick chamois skin used for a diaphragm, while for some of the more delicate algae it has been found advisable to use as low as 1 per cent. alcohol in the tube.

The strength of the alcohol in the jar can be kept up by adding to it from time to time some calcic chloride, which will in no way injure the alcohol. The jar should be tall enough to allow the cover to be kept on while the tubes are in position and thus prevent evaporation.

An apparatus of such a form with a dozen dehydrating tubes can be used for months without changing the alcohol. Other hardening agents such as picric, chromic, acetic, or osmic acids can be

used with equal success. Not more than 24 hours is necessary for dehydrating and hardening nearly all kinds of plant tissue.

The apparatus does away with the transferring of the tissues from bottles containing alcohol of different strengths, and since no such sudden transition occurs, the tissue is less liable to shrink. Many different materials may be used for a diaphragm and almost any speed of dehydrating can be obtained. The apparatus can be made in any size to adapt it for private or general laboratory work.

Picric Acid.

This is to be recommended as a hardening agent. It acts rapidly and energetically and should be used first in dilute solutions. About one-fifth per cent. is the proper strength. Tissue hardened in picric acid can be kept in 75 per cent. alcohol until needed. If a little glycerin is added to the hardening agent, the tissue will be less brittle.

Chromic Acid.

This agent can be used for hardening and with it the tissue requires the same treatment as with picric acid.

Osmic Acid.

This acid is very useful as a fixing agent and is often used for hardening. The tissue must not be kept in it long, as it will become blackened and brittle.

Hardening Fluid. See p. 17.

Other hardening agents are strongly recommended, but enough has been said to enable one to properly prepare tissue for such studies as may follow in this work.

CUTTING AND MOUNTING TISSUES.

The methods of cutting and mounting tissues are indeed numerous and only the more important ones will be reviewed.

Free-Hand Sectioning.

Many things can be prepared for study in this way. The tissue must be firm in order that it may not be crushed under the knife and yet not be too hard or brittle to prevent its cutting readily. The object to be sectioned should be held between the thumb and fore finger, while the razor should rest on the tip of the finger with the edge inward. Draw the knife, with the edge pressing against the tissue, across the finger keeping the thumb well below the line of cutting. Do not try to cut large sections but make them small and if need be wedge-shaped in order to get a piece thin enough for study. It is usually best to keep the tissue wet with alcohol or water to make the sectioning easy. Transfer the sections from the razor to the slide with a camel's hair brush.

If the object to be sectioned is quite small it can be placed between two pieces of elder pith or cork and sections made through these which shall include in them the sections of tissue. It is often very convenient to fasten the tissue with the cork, or if large enough, directly in a microtome, and section with a microtome knife or razor, keeping the object and knife wet as before directed.

This method is a very useful one for the transections of woody stems and firm tissues. These can frequently be softened without shrinking by leaving them for some time in glycerin, and in the case of the firmest ones, boiling for five minutes or more. Many small objects can be held for sectioning by placing them at once in

melted hard paraffine and cooling it quickly by immersing in cold water. The paraffine block can then be placed in a microtome and the object sectioned. This method will apply only to those objects that will not be shriveled by the high temperature of the melted paraffine.

For thorough, systematic study some method should be used that will enable sections to be made with accuracy, and if need be, in a series with a known definite thickness. The method should also provide something that can be infiltrated into the tissue and hardened to prevent crushing while being cut. The collodion and paraffine methods are the ones usually used for this purpose. Many modifications of both have been suggested but each method will be outlined to apply to the more general kinds of tissues. Experience will assist in modifying them for special cases.

Paraffine Method.

Much discussion has arisen in regard to the relative merits of the different paraffine methods, but the general differences are more or less of an unimportant nature and because of the different kind of tissues subjected to treatment. The most important methods are those of Mohl, (Bot. Gazette, Jan., 1888), and Schoenland (Bot. Gazette, July, 1887), while most of the others are modifications of these resulting from the experiments of different workers on the different classes of tissue.

The tissue to be treated is first hardened in chromic or picric acid or mixtures of these with other agents. As was suggested by Mohl, the acids act on the tissues in some way and make them much more penetrable to the infiltrating mass. Paraffine does not easily penetrate tissue treated with alcohol, yet many recommend it as a good hardening agent even under these conditions. A good mixture for hardening is made from equal parts of 1 per cent. chromic acid, osmic acid, 2 per cent., and acetic acid, 1 per cent. The osmic acid is very useful in many hardening agents for fixing the protoplasm, especially if it is desired to demonstrate karyokinesis, or cell division. Tissue should be kept in this mixture from 24 to 48 hours and then washed with running water 6 to 8 hours, after which it is placed for 12 hours successively in alcohol of 20 per cent., 40 per cent., 60 per cent., 80 per cent., and 95 per cent.,

strengths. A Schulze's apparatus is to be recommended for this operation. If alcohol has been used for hardening, the disagreeable process of washing can be omitted. The alcohol is now replaced by some solvent of paraffine, as chloroform, turpentine, clove or cedar oil, xylol, or benzol. Chloroform is readily miscible with paraffine, but is not very penetrating, and therefore requires a much longer time for clearing than some of the other solvents. Further it must be *entirely* driven off before sectioning, otherwise the paraffine will be too soft to support the tissue. Turpentine is recommended by Mohl, but this is often harmful to delicate structures. Cedar oil is perhaps the best of any of the agents for penetrating and clearing. Should turpentine be used, as Mohl recommends, the tissue is first placed for a few hours in a mixture of equal parts of alcohol and turpentine and an equal length of time in the pure agent, then after a few hours it should be removed, placed in a cold saturated solution of paraffine in turpentine, after which it is placed in a bath of equal parts of turpentine and paraffine, kept at the temperature of 30° to 40°C. In a few hours the tissue is transferred to pure paraffine, with a melting point of 50°C., and in this it is allowed to remain until thoroughly saturated with the imbedding mass. With cedar oil the object is transferred directly from the clearing agent to pure paraffine and left there until infiltrated. The time required for infiltrating any object depends upon the nature of the tissue. For some objects an hour is sufficient, while with others one or two days is required. After the object is infiltrated it is placed in a paper box and pure melted paraffine is poured over it. A convenient form of a paper box can be made as follows:

Take a piece of stiff paper of the proportions shown in Fig. 2 and fold inward along the lines A A and B B, one third of each side. Repeat the operation with the ends, folding them along the lines C C and D D. At each corner fold inward on a line bisecting the angle formed by the lines A A and B B, etc., allowing the crease to follow the lines E E, E′E′, etc. Then turn up the sides and ends until they are at right angles with the bottom, and bring back around the ends the portions projecting at the corners. Fold outward the portion projecting above the sides of the box and press it firmly against the end thus holding all the parts in place.

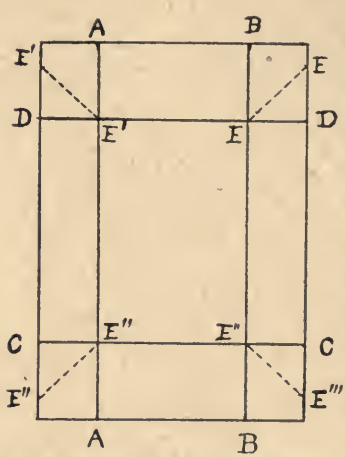

Fig. 2. Diagram to show the lines of folding in making a paper boat.

This box will be found most useful for various purposes in the laboratory. The object in the box can be arranged by the use of hot needles, while the paraffine is yet in a liquid state. As soon as a film is formed over the paraffine, it is plunged into cold water to harden quickly and thus prevent the mass from becoming filled with air spaces. After the paraffine has become hard it can be readily removed from the box and fastened in a microtome for sectioning.

Several different forms of microtomes are highly recommended. Minot's is certainly one of the best. A very inexpensive well microtome will answer the purpose. In case the latter is used the block containing the object should be cut down until it will fit in the well where it is fastened by pouring over it pure melted paraffine. It is best that a microtome with movable jaws be used in order that the position of the object can be changed to vary the angle of sectioning. This is especially important in longisections of roots or stems. If longisections of a root are being made or the whole of an object is to be studied, it is advisable that a series be made and only those need be mounted which contain the structure desired. In order to stain and clear the sections it is necessary that they be in some way glued to the slide. This can be accomplished in several ways. Those methods to be especially recommended are as follows: With a camel's hair brush spread over the slide a very thin layer of a mixture of equal parts of clove oil and 2 per cent. collodion. Place the sections on the slide and press them gently against it with a brush or any soft object. The preparation is then put in an oven with the temperature at 50° C. for 20 minutes, or until the paraffine has melted and the clove oil evaporated. The sections will then have become fastened to the slide and can be stained and washed without danger of loosening. The same result can be more quickly obtained by heating the slide *very cautiously*

over a flame until the odor of the clove oil has disappeared. Care must, however, be taken not to shrivel the tissue by overheating it.

A better method of fastening the sections, is to albumenize the slide by soaking it a short time in a mixture of egg albumen 1 part, water 200 parts, and then allowing the slide to drain until dry. A number should be treated at one time and kept on hand until desired for use. With these slides, by warming the sections until the paraffine melts, the preparation will flatten out and adhere with great tenacity to the albumenized surface. The albumen will not be effected by staining.

After the sections are fastened, the slide is placed in turpentine, or better, in xylol to dissolve away the paraffine. This will usually be accomplished in 15 to 20 minutes, when the object is washed with water and is ready for staining. The stain to be used and the method of applying it depends largely on the nature of the tissue and the part one desires to bring out. For general studies haematoxylin works well and with eosin makes a good double stain. If the haematoxylin is used, the slide need be left in it but a few minutes, when it is removed and washed thoroughly with water to take out the surplus stain, dehydrated with 95 per cent. alcohol, cleared for a few minutes in clearing mixture, and mounted in balsam. If the tissue is to be stained in toto, before imbedding, Mohl recommends that it be taken from the 60 per cent. alcohol, while being hardened, and placed for 24 hours in a solution of alum carmine, after which the hardening can continue. With this treatment, when sectioned, it is only necessary that the paraffine be dissolved away, the tissue cleared with a clearing agent, and mounted in balsam. With haematoxylin or alum carmine the cell wall stains well and the nuclei show very clearly. In double staining, if eosin is applied, the sections need be left in it but 20–30 seconds. If glycerin jelly is to be used as a mounting medium, the sections can be mounted directly after washing off the surplus stain with water.

During the sectioning it may happen that some of the parts of the section will loosen as they are being cut away. In this case they may be held together with collodion applied in a 1 per cent. solution with a camel's hair brush. This is painted over the tissue just before the section is cut, it dries quickly and hold all the parts

in place, while it does not in any way interfere with the staining. It is advisable that all paraffine sections be mounted in balsam. The use of the paraffine method is recommended for the firmer tissues that cannot be held in place by the collodion.

The objections to the paraffine method are that heat is employed in infiltrating and this is injurious in some degree to most tissues. Further the length of time required to get the tissue in condition to section is quite extended, and this is often an important consideration. The operations requiring paraffine are not as clean in the hands of most students as would be desired.

The Collodion Method.

This method is now coming into general use for nearly all kinds of plant tissue. For the use of collodion for infiltrating we are indebted to Duval, who first published his results in the Journ. de l'Anat., 1879, p. 185. A little later Merkel and Schiefferdecker suggested the use of celloidin, which is merely a patent collodion. Some discussion then arose regarding the relative merits of each, but it is generally agreed that one has little or no advantage over the other.

The method as applied to plant tissues is a comparatively new one and many modifications of it are at present recommended by various workers.

The following directions are in the main taken from a report of the author on the method, made before the Am. Soc. of Microscopists and published in their proceedings in 1890.

The tissue to be treated is first dehydrated and hardened in alcohol. For this purpose a Schulze's apparatus is of the first importance, in fact it has been found that some tissue can be hardened in no other way without shrinking. With its use, from 12–24 hours is sufficient for hardening and dehydrating any plant tissue. The material is taken from the dehydrating apparatus and placed in 95 per cent. alcohol for one hour, to insure complete dehydration. There is then poured over it a 2 per cent. solution of collodion. In this it is allowed to remain from 12–24 hours, depending on the nature of the tissue, 24 hours being enough for the very firmest. It is then transferred for the same length of time to a 5 per cent. solution; or the 2 per cent. solution may be allowed to

evaporate until it is of the consistency of the former. After this, it is taken out and arranged in position on a cork or block of wood of convenient size to fit in the jaws of the microtome. By means of a camel's hair brush the material on the cork is covered with successive layers of collodion until it is quite enclosed in the mass. Allow each coat to dry slightly in the air before applying the next. After the tissue is covered it is placed in about 80 per cent. alcohol until hard enough to section. Much difference of opinion exists regarding the proper strength of alcohol to use for hardening the collodion, but 80 per cent. answers very well, and the tissue can be kept in it a long time without deteriorating. After a few hours the collodion will be firm enough for use. Sections of small or delicate objects can be cut by allowing them to harden in a block of collodion and then carefully clamping it directly in the jaws of the microtome. For sectioning, any sliding microtome will answer, but one especially adapted for the purpose will enable the object, which can be clearly seen through the collodion, to be inclined in any desired position and sections taken in any plane. It is also necessary that the sections be removed with a long sweeping cut, since a direct cross-cut would tear them. The sections should be covered with alcohol while being removed and then floated from the knife to the slide. (See P. A. Fish's modification, Proceedings Am. Mic. Soc. Aug, 1893.) The slower the section is cut, the better it will usually be. Serial sections can be arranged in their proper place on the slide. For fixing to the slide, blow some dry ether vapor on the object (Fig. 3), or add a drop of ether to the side of the preparation. The ether dissolves the collodion and fastens the sections in place. The preparation is then washed with water, stained, the surplus stain washed off with water, the sections dehydrated with 95 per cent. alcohol, cleared, and mounted in balsam.

For clearing, the carbolic acid and turpentine mixture is to be recommended. It clears quickly and does not injure the most delicate tissues. For staining, one must use that which seems best adapted for their purpose, but for general study, haematoxylin seems especially adapted for collodion sections.

Some difficulty may arise in cutting sections that have in them free parts. It sometimes happens that they become detached from the collodion and float away. In this case, the section can be

Fig. 3. Ether wash-bottle for blowing ether vapor upon collodion or celloidin sections to fasten them to slide. The tube of calcium chloride ($CaCl_2$) is for dehydrating the ether vapor.

collodionized as first suggested by Dr. Mark. This is done by coating the tissue before each section is cut, with a thin coat of one per cent. collodion, using a camel's hair brush for the purpose; then draw the knife across the tissue very slowly, keeping alcohol dripping on it while the section is being cut. In this way beautiful sections can be obtained of material with loose parts, where all will retain their proper position. Care should be taken that none of the sections be cut before collodionization, for although it may not always be necessary to keep the parts in place, yet it is a safeguard against their displacement.

The method given is found to work admirably on very delicate meristematic tissue. No heat being required, the most delicate of tissues will not shrink. The shortness of the method commends it for general use. Two days, or even less, is sufficient to go through the whole operation of hardening, infiltrating, and sectioning, nearly all kinds of plant tissues.

The sections after being cut can be easily handled with a camel's hair brush without fear of breaking.

In the case of delicate tissues, like fern prothallia, or the apical cell of Nitella or Chara, some little variation is made from the regular method and a detailed description of the process may be of value. (The Microscope, Nov. 1893.)

The material is first placed in 10 per cent. alcohol in a dehydrating apparatus and allowed to remain for 24 hours, when it is taken out and placed for one hour in 95 per cent. alcohol to insure complete dehydration. After this the tissue is placed in a 1½ per cent. solution of collodion and allowed to remain in a tightly corked vial for 12 hours. The cork is then removed, and by slow evaporation of the ether and alcohol, the collodion will thicken. When it is of the consistency of ordinary glue, the preparation is poured out of the vial, with the collodion, into a paper boat, of the kind used in paraffine imbedding, or an ordinary watch glass will answer the purpose. The thick collodion is then poured over the tissue and allowed to harden in the air until a firm film has formed over the surface. After this the mass is placed in a jar of 85 per cent. alcohol and allowed to remain from 5–6 hours until the collodion is quite tough. Then with a thin knife cut out a block of collodion containing the tissue inside. The block can be placed in any desired position on the end of a cork and held while thick collodion is poured over, until it is covered. Each coat as added should be allowed to slightly harden before applying the next, until the operation is completed. After the whole has become firm in the air, it is placed in a jar of 85 per cent. alcohol where it should remain 6 to 8 hours. The operation of sectioning and mounting is the same as outlined for the firmer tissues.

In order that the sections may be all arranged the same side up, the block of collodion, which should always be trimmed at the top, can be cut with a notch near one corner, and the notches all arranged with the same relative position on the slide.

It will be found that with this method perfect serial sections of any desired thickness can be obtained from objects which are not more than one layer of cells thick, and thus render the preparation of delicate tissues but little more difficult than that of the firmer kinds found in ordinary stems and roots.

Many substances for infiltrating have from time to time been suggested, and have met with varying success. The more impor-

tant ones tried are gelatin, gelatin soap, paper, wax, gum arabic, and paraffine mixtures, but it is not necessary to outline other methods here as the few described in detail will enable the student to prepare material for any work suggested in this manual.

STAINING AGENTS.

Only a few of the more important staining agents will be mentioned, and directions given for their general use.

Ammonium Carmine.

This stain is best prepared, as suggested by Hartig. Dissolve a little carmine in water until the mixture has the consistency of paste. Add to this a little strong ammonia and evaporate the whole to dryness over a water bath. The resulting powder dissolved in water is used for staining.

Alum Carmine.

Make a concentrated solution of alum and add to it enough powdered carmine to give it a deep color, (1 gram to 100 c.c. of alum solution). Boil for 10 minutes and, when cold, filter. This agent is much used and is often valuable as a selective stain. It colors pure cellulose cell walls a bright red, but does not effect those that are lignified or suberized.

Picro-Carmine (Gage).

Twenty grams of picric acid are dissolved in 200 parts of water, and mixed with 5 grams of carmine in 250 c.c. of strong ammonia. Stir the whole thoroughly and evaporate to dryness. Dissolve the residue in 700 c.c. of water. All of the carmine stains are very useful, easily handled, and quite selective. Picro-carmine turns protoplasm a yellowish red.

Eosin.

This is a valuable general stain, as it has a great coloring capacity. It stains nucleus and cell wall readily and is much used in double staining. It can be applied either in an aqueous or alcoholic solution; 1 gram of eosin to 100 c.c. of water is a very convenient strength.

Haematoxylin.

This coloring agent can not be recommended too highly. It has a very wide application and gives uniformly good results. The stain is made by adding a concentrated solution of haematoxylin crystals in alcohol, cautiously, to a 3 per cent. aqueous solution of alum, until a medium purple color is obtained. The solution becomes darker and better by standing a few days, but deteriorates after a time and will require filtering often. As suggested by Prof. Gage the addition of chloral hydrate and proper sterilizing of the constituents of the stain during its manufacture greatly increases its keeping power. (Proc. Am. Microscopical Society, Jan. 1893).

The old stain, if kept in a cool place, is very valuable in staining meristematic tissue. Haematoxylin stains the nucleus a deep blue or purple and is to be recommended for all general work. It is sometimes used in staining bacteria and with other stains in double staining.

ANILINE COLORS.

These colors have of late been very useful in furnishing stains for histological work. To them we owe much for the present valuable and convenient methods of staining. Only a few of the more important agents can be included in this outline.

Methyl Violet.

An aqueous solution of this is much used in staining bacteria. It is also valuable as a selective stain for many plant tissues.

Methyl Green.

An aqueous solution with 1 per cent. of acetic acid is used for staining the nucleus and chlorophyll grains. As a double stain it is often used on transections of stems in connection with eosin. Iodine green is, however, preferable for double staining.

Aniline Blue.

This stain is much used in connection with the staining of bacillus tuberculosis, but is also very satisfactory as a stain for sieve plates and sieve tissue. Cellulose takes a blue color while the sieve plates become azure. Sections treated with this color are liable to fade after a time. The stain is good in double staining.

Magenta.

Magenta is used both as a general and selective stain. It should be applied in an aqueous solution, containing a little acetic acid. The tissue will need to be left in the solution for some time, before the proper depth of staining is secured.

Picric Acid.

This is a very convenient and useful ground or general stain and is usually applied in weak alcoholic solutions. It must be borne in mind, however, that picric acid will wash out to quite an extent many of the aniline colors, but in use with haematoxylin, borax, or alum carmine, it makes a most excellent general stain. In connection with hydrochloric acid it will readily wash out the carmine colors.

Silver Nitrate.

In a dilute alkaline aqueous solution this is often used as a test for living protoplasm, since it colors it black, while dead protoplasm remains unchanged. The reaction is very delicate and positive. Tannic acid also colors less dilute alkaline solutions black, while cells containing glucose are colored brown. As a general stain the action of silver nitrate is too uncertain for positive directions.

CLEARING AGENTS.

The function of a clearing agent is to make the tissue transparent by penetrating into all parts of it.

It must be a liquid of high refractive index and miscible with balsam, as well as having the power to drive out all of the alcohol. The agents employed are very numerous, but a successful one should replace the alcohol quickly and yet not destroy the tissue by shrinking. The chief clearing agents are the essential oils.

Cedar Oil.

This oil is a very good clearing agent but is quite slow in its action; however, it does not shrink the tissue nor fade aniline colors.

Clove Oil.

This is one of the best clearing agents. The clove oil on the market is usually impure and not suitable for use. The pure oil can be obtained only from reliable dealers. As a clearer, it penetrates tissue very readily and clears most thoroughly. It has a very high refrative index.

Collodion is dissolved by this oil, and it is therefore not safe to use with collodion sections unless proper precautions are taken to prevent the displacement of the disconnected parts.

Tissue that remains in clove oil any length of time is liable to become brittle, and this is sometimes very helpful in minute dissections.

Aniline colors are often faded by the action of this clearer.

Oil of Origanum and Oil of Sandal Wood.

These are both to be recommended as clearing agents, but for many tissues are not wholly satisfactory.

Turpentine.

Turpentine is much used for clearing paraffine sections, as it dissolves out the paraffine and clears the sections at the same time. It is very liable, however, to cause sections cut in alcohol or collodion to shrink.

Carbolic Acid and Turpentine.

This is to be recommended as the cheapest and at the same time the most satisfactory of all clearing agents.

The mixture is made of 3 parts of turpentine and 2 of pure carbolic acid. It clears equally well paraffine, collodion, or alcoholic sections, and needs but a few minutes to thoroughly permeate the tissue. It is best to filter the mixture through cotton to remove all particles of dust. The carbolic acid gives the hands an unpleasant feeling and they should therefore be kept free from it.

MOUNTING MEDIA.

Aluminium Acetate.

In a saturated aqueous solution this salt forms a good mounting medium for many delicate organisms. Most algae can be preserved in this way without any deterioration, while such objects as the young prothallia of ferns can be kept without shrinking or losing much of the brightness of their chlorophyll or protein granules. Like all liquid mounting media it must be used in a cell, and the slide should lie for 24 hours before sealing.

Balsam.

Balsam forms a most excellent and substantial mounting medium. Sections mounted in it should be free from water and this can be easily brought about by the use of alcohol. It is best to begin with alcohol of moderate strength (50 per cent.), and gradually increase it until the tissue is taken from that of 95 per cent. strength. Before mounting, the sections must be cleared of alcohol by the use of turpentine, chloroform, oil of cloves, or better, a mixture of 3 parts of turpentine and 2 of pure carbolic acid.

Balsam is prepared by evaporating over a gentle heat common commercial *Balsam of fir* until the volatile oils have been driven off and the residue becomes brittle. The portion that remains is then dissolved in cedar oil, xylol, or chloroform, and filtered through glass wool in a paper funnel. The medium should, at the ordinary temperature, be of the consistency of a thick syrup. Care must be taken to keep air bubbles from balsam. If, however, they get under the cover they can be driven out if the slide be left in a warm place for a few hours.

Although not absolutely necessary to seal the cover glass of balsam mounts, it is, nevertheless, a good precaution, as the cover may crack away from the medium and the section be displaced. The balsam should always be kept in a glass-stoppered bottle, and applied to the slide from a pointed glass rod.

Carbolic Acid.

Carbolic acid in a 1 per cent. aqueous solution is used as a mounting medium, also carbolic acid crystals in glycerin, for some of the firmer tissues. These agents have a tendency to make the sections clear or faded.

Calcic Chloride.

About one part of this salt to two parts of water is a good mounting medium for many tissues. A little camphor should be added to the solution to preserve it.

Glycerin Jelly.

This is extensively used as a mounting medium for small and delicate objects, which might be injured by clearing for balsam. Glycerin jelly mounts will, after a time, become transparent, unless they have previously been stained with a permanent stain. Care should be taken in mounting sections to prevent air bubbles getting under the cover glass, since they do not disappear as in balsam mounts. Glycerin jelly mounts should be sealed as soon as cold.

Many formulae for the preparation of this mounting medium are in use, several of which seem to be equally good. Kaiser's jelly is easily made and keeps well. It is prepared by soaking one part by weight of best French gelatine in six parts of distilled water for two hours; 7 parts of glycerin are then added, and 1 drop of concentrated carbolic acid for every gram of the mixture. Warm 10–15 minutes, with constant stirring, until the mixture becomes clear, and then filter while warm though wet glass wool in a hot water filter. The jelly will require warming before use. The mixture keeps well for years and is a very convenient mounting medium.

Glycerin.

Glycerin is often used as a permanent mounting medium and, with proper precautions, gives satisfactory results. To prevent

shrinking, sections to be mounted in glycerin should first be placed in a mixture of equal parts of glycerin and water and allowed to remain a little time, when stronger glycerin is added at intervals, until the section is thoroughly permeated with the pure agent. As this is hygroscopic, the mounts must be sealed at once as they will readily absorb a quantity of water sufficient to dilute its strength appreciably. The cover can be sealed first with a little glycerin jelly, and, after this has hardened, with shellac or asphalt.

Glycerin is a very good mounting medium for studying fresh tissues, as it evaporates very slowly. Sections mounted in it can be kept for examination for some time. Glycerin, like glycerin jelly, has the property of making sections clear or transparent. They should therefore be treated with a permanent stain. Only the purest commercial glycerin should be used, and this must be kept in a tightly corked bottle, otherwise, it will absorb a sufficient quantity of water to render it almost useless as a mounting medium.

Glycerin and Acetic Acid.

A mixture of equal parts of glycerin and acetic acid is a very convenient mounting medium for many kinds of tissue. Especially is this true with some of the fungi. In the preparation of this mixture, pure glycerin and concentrated acetic acid should be used.

King's Mounting Medium.

This is good for many fresh-water algae, and like all fluid mounts it must be used in a cell. It can be secured of dealers in microscopical supplies.

Water.

Water is not infrequently employed as a mounting fluid. To preserve the mounts from deterioration a little camphor should be added. Water is often used as a medium for the studying of fresh tissue; but it should be borne in mind, that it may change the nature of the tissue materially, owing to the osmosis between the cell contents and medium. This can be prevented by adding some substance to the water to make its density equal to that of the tissue, or cell contents. Salt is sometimes used, but is by no means efficient.

CEMENTS.

The number of cements and varnishes is so numerous that one is at a loss to know just what is best to use, but the general characters of some of the more common ones will be briefly noted.

Gold Size.

This cement can be secured of dealers in microscopical supplies and is certainly to be well recommended. If used for balsam mounts, it is best to first ring the cover glass with a coat of shellac.

Shellac.

Shellac is certainly very convenient and seems to be quite durable. It is prepared by dissolving solid shellac in alcohol until the solution is of a medium oily consistency. A little aniline dye can be added to color the mixture to one's fancy, also a few drops of oil should be used to prevent the cement from cracking. The mixture is applied, as are all sealing mixtures, with a small brush and, preferably, by the use of a turn-table.

Ball Cement and Asphalt Varnish.

These are among the very best of cements and can be obtained from the regular dealers in supplies.

White Zinc Cement.

This cement is much used by microscopists for making cells. It is very hard and often shows a tendency to crack.

Many very good cements are on sale by reliable dealers, and one has but to convince himself of the relative merits of a few of the more important ones, when he will be able to settle upon some special one well adapted to his purpose.

SERIAL SECTIONING.

It is often very desirable and indeed quite necessary that the whole of an organ be studied systematically, but to do this requires that the parts not only be placed in a condition to be observed, but also, that the arrangement of parts as sectioned be so systematic as to show the relation of each section to the whole. This can be accomplished by making what are known as serial sections of the object. In this case it is necessary that the sectioning be done in a microtome in order to preserve a uniform thickness. The sections as cut, should be arranged on the slide close to each other and in the same order as they are removed from the object. They should also occupy the same position with reference to the relation of each section to the whole.

With the paraffine method when "ribbon sections" are cut, it is only necessary to break off pieces of ribbon and lay them alongside of each other in the order in which they are removed.

With the collodion method as the sections are cut, they should be taken up with a camel's hair brush and transferred to the slide. After being arranged in position, they should be sealed by blowing over them a little ether vapor. The slide should be kept constantly wet with alcohol to prevent the sections from drying or shriveling. The arrangement of sections on the slide should be uniform at all times. A very convenient order is to begin in the upper left hand corner and place the sections under each other in a row along the longest axis of the slide. After the row has reached to within $2\frac{1}{2}$ c. m. of the opposite end, a new row is begun at the right of the old one, and at the top of the slide; $5 \times 2\frac{1}{2}$ c. m. cover glasses will be required.

When the sections are to be designated by number, they should be numbered in the order in which they are placed on the slide. Serial sectioning is very important in any thorough investigation, since it places before the observer *all* of the object to be studied, in condition for careful searching, enabling one to trace the course of any part in every direction through the object. Serial sectioning certainly saves much time and prevents many misconceptions that might otherwise arise from the examination of a single preparation.

DOUBLE STAINING.

It is often desirable, in order, to bring out more clearly some part of a specimen, to stain different portions of it with separate colors. This is very easy to bring about if the proper stains are employed. For example, the xylem of a fibro-vascular bundle can be stained one color and the phloem another. These results are very important in research as one is enabled to recognize in this way similar tissues in different parts of a specimen where otherwise they might be somewhat difficult to distinguish. Certain stains will color one part of a tissue or special part of a cell, and yet not effect in any way other portions.

The combinations of stains that can be used for this purpose are very varied, and the results in the case of some quite uncertain to predict, consequently no general rule can be laid down in regard to the kind of tissue any stain will invariably act upon.

With regard to the treatment, in general the selective stain should be applied first, and in dilute solutions. The sections should then be thoroughly washed and the general stain applied, the tissue washed again and mounted directly in glycerin jelly or dehydrated, cleared, and mounted in balsam. Glycerin jelly is perhaps a better mounting medium for double stained tissue, since the alcohol and clearer are often injurious in their bleaching effect. Some combinations of stains whose effects can be depended upon are haematoxylin and eosin or picro-carmine, iodine green and eosin, carmine or haematoxylin with picric acid, and methyl green and eosin.

It is often necessary to use a mordant to fix the stain, in which case a saturated solution of alum can be applied to advantage. A little experience with double staining will enable one to use it in a way that will be of great assistance to a better interpretation of the character of many tissues.

FLUID MOUNTS.

Mounting in Cells, Etc.

It is frequently desirable to mount small objects in such a way as to preserve them without crushing or mutilation. In this case the cover glass must be supported in order that it may not come in contact with the preparation. This is accomplished by mounting the object in a cell, which can be made in various ways, depending on the nature of the object to be mounted. If the specimen is very small and is to be preserved in balsam or glycerin jelly, it may suffice to place around the object a few pieces of broken cover glass to keep the preparation from being crushed by the cover. Seal as in other mounts. If the object to be mounted is quite large, a deep cell must be made.

The nature of the material of which the cell should be constructed must depend on the character of the mounting agent, that is, the material of which the cell is made must not be of a substance in any way acted upon by the mounting fluid. For many mounting media, cells made of shellac are very convenient, and quite durable.

To make the cells, place the slide on a turn table and when the table is revolving, touch the slide with a small brush dipped in shellac. A ring is the result. After the shellac has dried, another ring should be made on the top of the first. Allow this to dry and repeat the operation until the cell is of the desired depth. Several of these cells should be prepared and kept on hand until they may be needed. Before using, paint over the top a *very light* coat of *thin* shellac. The object to be mounted should be placed in the center of the cell, and the latter filled completely with the mounting

medium. To place the cover glass in position, rest one side of it on the edge of the ring and lower it slowly over the object, allowing the surplus mounting fluid to be forced out on one side. Press the cover firmly in position. Before sealing fluid mounts, fasten the cover in place by applying three or four small drops of sealing mixture at the edge of the cover glass at different points. As soon as dry, these hold the cover in place and allow the mount to be permanently sealed. Cells may be made of glass, zinc, bone, or celluloid rings which can be secured of any regular dealer in microscopical supplies; or rings can be cut from sheet lead, tin, paper, or wax, and fastened in position with shellac or marine glue applied to the base of the cell and then held firm, by the ring, against the slide until dry. Seal around the outside and when ready for use, apply a light coat of sealing mixture to the top, to hold the cover-glass in place. When the object is mounted, seal the whole preparation as in the case of ordinary mounts.

Objects can be easily mounted dry in cells, in which case they should be held in position by fastening them to the slide with a little glycerin jelly, collodion, or gelatine. Fluid mounts should be examined from time to time to repair any that may not have been perfectly constructed.

EQUIPPING OF LABORATORY.

In the equipping of a laboratory, one must necessarily be controlled largely by the material and funds at their disposal, but a few suggestions may serve to lighten somewhat the care of overseeing so much manipulation.

Each student should be provided with a case made by boring two rows of holes about 45 m. m. in diameter in a block of wood 30x14 c. m. and 45 m. m. thick. In this should be placed bottles containing the more general reagents and stains that will be needed frequently in the work. It is suggested that the set consist of Iodine, Acetic, Sulphuric, and Hydrochloric acids, Glycerin, Potassic Hydrate, Eosin, Haematoxylin, and Clearer. Another case containing reagents and stains of a more special nature can be placed on a center table easily accessible to all. The desks should all be equipped with wash bottles of alcohol and water, and a general supply of the same agents should be located in a convenient place in the laboratory. These arrangements will prevent the student from being compelled to walk about searching for some reagent or supply. Cases should be provided for containing the general store of chemicals and glassware. The books should also be in a convenient place easily accessible to all.

It is much more desirable that the microtomes, hardening and infiltrating apparatus be on a special table which should also be supplied with all the stains and reagents necessary for mounting the sections. A convenient form of a waste vessel over which the sections can be treated is made by fastening, with sealing wax, to a tray or dinner platter, glass rods, parallel and about 4 c. m. apart. The slides with the sections can be laid on these rods and treated

with the various reagents, which can be washed off directly into the tray beneath and thus prevent table or hands being soiled by the stains or clearer. It is very convenient to have a small supply of alcohol and water in bottles elevated at a little distance on a shelf, and provided with a siphon having rubber connections below to enable it to be used in various parts of the table. The orifice of the glass tip should be small and the rubber tube provided with a pinch cock to regulate the supply at pleasure. This arrangement will be found very convenient to keep knife and tissue wet while sectioning tissue imbedded in collodion, and also for dehydrating and washing the sections on the slide.

For the clearer, a wash bottle should be provided with an enlargement at the inner end of the exit tube filled with cotton, thus preventing any particles of dirt from getting into the clearer used on the slide. The enlargement can be made by cutting off from the end of a glass tube, just small enough to enter the mouth of the flask, a piece about 5 c. m. long. Close one end with a cork and pass through a hole in this exit tube. Then fill the piece of tubing loosely with cotton. A similar bottle will be found very useful for the haematoxylin. (Fig.3.) Many devices will be suggested in the laboratory from time to time and materially lighten the burdens incident to having the charge of so much laboratory instruction.

COLLECTION AND PRESERVATION OF MATERIAL.

Much of the material for work in histology must be collected during the summer, or at times when it cannot be used at once, and is therefore to be kept stored away until needed for study.

Some difficulty must necessarily arise in the proper preservation of such material, but if special precautions are taken it can easily be kept in good condition for histological purposes. The methods of treatment vary with the nature of the material.

All soft tissue collected, as, for example, leaves, herbaceous stems, etc., should be placed at once in 40–50 per cent. alcohol and hardened in the usual way by the use of a Schulze's apparatus, or by transferring successively, for 24 hours in each, to 50 per cent., 67 per cent., and 75 per cent. alcohol, in which it can be kept a long time without deterioration. The softest tissues such as are found in algae, etc., should be placed first in about 10 per cent. alcohol and hardened by the use of Schulze's apparatus, or by transferring, successively, for 24 hours in each, to 20 per cent., 30 per cent., 40 per cent., 50 per cent., etc., to 75 per cent. alcohol, where they may be kept as in the case of the firmer tissues. The material thus hardened when ready for use can be dehydrated with 95 per cent. alcohol, infiltrated with 2 per cent. and then 5 per cent. collodion. In the latter solution it may remain indefinitely without shrinking. If the tissue to be sectioned is kept on the cork in alcohol, it will in time become discolored by the tannin of the cork.

The tissue of each sort should be trimmed until only such parts remain as are needed for sectioning. These should be care-

fully tied up in pieces of bibulous paper, with the labels written in India ink or pencil, inside. In this way, many different things can be kept in the same jar and thus economize room and solutions. If the material is to be preserved in thick collodion, the stopper of the vessel in which it is placed should make the bottle air tight and should also be held in place in some way, otherwise the evaporation of the ether may force it out and ruin the material by the hardening of the collodion. If it is desired that the tissue be kept on blocks in alcohol any great length of time, in order that it may be ready for use at once, hard rubber rods sawed into convenient lengths of about 2 c.m. may be substituted for the cork. As the alcohol does not effect the rods, tissue can be kept in this way any length of time without deterioration. The rubber rods can be obtained of the Educational Supply Co., Boston.

Blocks of hard wood have also been suggested for the same purpose.

In collecting material to preserve for class-room work, much care should be taken to prevent confusion of labels, etc., and all important data should be included with the notes on each study.

THE MICROSCOPE.

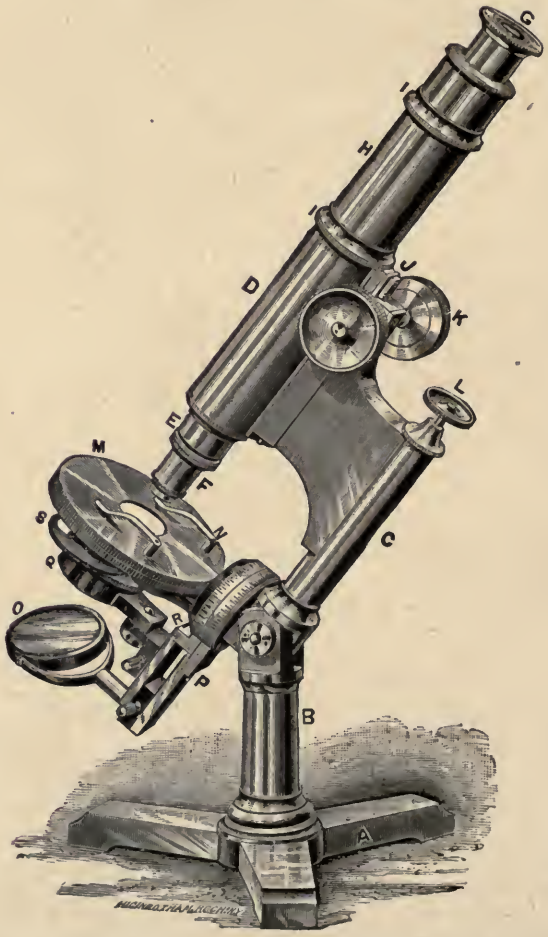

Fig. 4. Microscope, with the parts lettered to correspond with the description.

A. **Base** of the instrument and part that forms its entire support. This may be in the form of a horse-shoe or a tripod, supporting at three points. This condition is desirable, as greater solidity is thereby given to the instrument.

B. **Pillar.**—This is the upright support from the base and usually has a joint at the top, by means of which the instrument may be inclined.

C. **Arm.**—This carries all of the remaining parts of the instrument.

D. **Body.**—This is the tube holding the optical parts of the instrument that are above the stage. The raising and lowering of these is controlled either by a gearing or by friction of the outer stationary part with the inner movable one.

E. **Nose-Piece.**—A small revolving part fastened to the lower end of the body and forming an attachment for the objectives.

F. **Objective.**—This is the lower of the lens combinations above the stage and is usually screwed into the nose-piece. Most microscope makers use a uniform size of thread and this is known as the **society screw**. The function of the objective is to form an image of the object.

G. **Ocular.**—The part holding the upper combination of lenses, and fitting into the upper end of the body. The function of the ocular is to magnify the image produced by the objective.

H. **Draw-Tube.**—This forms the inner part of the body and moves in an outer sheath. The length of the body may be varied by the adjustment of the tube in its collar.

I. **Collar.**—A ring fastened to the upper end of the body and forming a sleeve for the draw-tube.

J. **Course Adjustment.**—This is used for raising and lowering the body. It is provided with two large milled heads (K), which revolve a pinion that acts upon a rack, and controls the working of the adjustment.

L. **Fine Adjustment.**—Used to raise or lower the body slowly through short distances, in this way obtaining the exact focus. It consists of a milled head with a screw that acts upon the body of the instrument.

M. **Stage.**—This is firmly attached to the pillar and is for the support of the object during examination. Sometimes a

movable slide carrier is attached to the stage. The more expensive instruments are often provided with a mechanical movement that enables the object to be carried in any desired direction by simply turning two screws located above or at the side of the stage.

F. **Clips.**—These are to hold the glass slide, on which the object is mounted, firmly against the stage.

O. **Mirror.**—This is one of the optical parts below the stage and is for the purpose of illuminating the object, either by throwing light through it, or on it from above. One side of the mirror is usually plane and the other concave.

P. **Mirror Bar.**—A bar attached to the arm and carrying the mirror. It can usually be swung from side to side to vary the angle of illumination.

Q. **Sub-Stage Ring.**—Used to support either the diaphragm or some of the optical parts that are used below the stage. It is often attached to the latter but in the more perfect instruments is borne on a separate bar.

S. **Diaphragm.**—This is a disk provided with numerous apertures, of various sizes, and can be turned to regulate the amount of light coming from the mirror to the object.

Field of the Microscope.—This is the clear area seen by looking into the microscope, and should be perfectly circular, when the instrument is properly lighted.

METHODS OF STUDY.

It is very important that the student follow from the beginning a system of work that will enable him to utilize the experience of those who may have had years of training and have learned the roads to uniformly good results. A few suggestions may not be out of place.

Care in Observation.

Avoid making hasty conclusions even though the appearances seem to warrant them. Do not consider as proven a condition that can be seen but once and then under difficulties. In important cases, always verify results by trying the study again with other material so that there can be no doubt as to the exact state of things. Never substitute an opinion or inclination for a fact even though it often involves a disappointment, better that than error.

Selecting Material.

Many of the troubles and difficulties in the way of a proper study can be overcome by taking due precaution in the selection and preparation of material. Too often a section is carelessly made with the hopes that it *may show something* desired, but it is better always to select the material carefully, then section and mount with all the proper precautions.

Directions for Drawing.

It is necessary that the student study and thoroughly understand the tissue under examination before any attempt is made to reproduce it, otherwise, after the drawing is finished errors in it

will probably be found, and it will not represent the true relations of parts. Do not draw everything that can be seen, but place on paper enough to represent accurately the general outline and minute structure of the object that is being studied. Let the relations of the parts drawn be so clearly indicated that a correct picture of the object can be perfectly formed in the mind from the drawings. The first drawing should usually be an outline sketch of the whole object that is being studied. Then should follow a more detailed drawing of each particular part as examined, and lastly, the minute structure of any special tissue or cell. It is true that such detail will require much time but practice will soon reduce this and make the work seem very easy. It is not best to shade any of the drawings since it may obscure the parts that should be kept prominent. Often in studying the minute structure of some organ, a single section will not give a complete outline of the whole part that is to be represented, in which case, it should be made up from the several sections that will best show the parts desired. Be certain, however, that the relations of parts are thoroughly understood and correctly represented. One is often inclined to make the drawings too small but this should be guarded against, and the sketches made of sufficient size to enable the parts to be clearly seen without close scrutiny. The drawings can be made free-hand or by the use of a camera lucida. The latter method is necessarily the more accurate but lacks much of the element of good training given by the former. If the drawings are made with a camera lucida, much of the detail will need to be filled in free hand, but the general outline can be made with great ease. The best camera lucidas are of the Abbe pattern which can be used without tilting the tube of the microscope. With it, some difficulty may be experienced in seeing the object and pencil point at the same time, but this can be overcome by having the paper equally illuminated with the object under the microscope. Other camera lucidas may be used but it is not necessary to outline the working of each in these directions. With the Abbe camera lucida, be certain that the drawing paper is at right angles to the axial line as reflected from the mirror, otherwise the drawing will be distorted. The variation a from perpendicular with the table can be determined by the use of a semi-circular protractor, which will give the

angle to which the mirror is tilted. The drawing paper should be raised at an angle with the table twice as great as the mirror is depressed below 45°. The drawing board should be made with a hinged part that will support the paper and yet allow it to be raised to any desired angle. The magnification of the drawing should be determined and indicated underneath it; (x300) indicates that the drawing is magnified three hundred diameters. Free-hand drawings should be made with special reference to exact proportion, in order that every part may have the same magnification. Pencil drawings should be made on good drawing paper and with sharp-pointed drawing pencils, which had better be of two degrees of hardness, one being quite hard and the other medium soft. The former can be used for detail work and the latter for general outline. The objection to pencil drawings is that they blur by rubbing and are not as clear as those made with ink. It is better to outline ink drawings at first with a pencil and then retrace and fill in the detail with the pen. The different parts of the drawing should be named at the side and the name connected to them with a dotted line. With a little care, this can be done without trouble, and the drawing book will look neat and be easily interpreted. Where any part of an object is to be repeated several times in the same drawing, it is only necessary to outline the parts and indicate the repetition. Also where there is to be shown a large mass of cells of uniform nature, the whole should be outlined and a few cells drawn to indicate their general character.

Next in importance to the drawing is the description, and this must not be neglected. It should always include a careful outline of the nature and intent of the study, together with a full explanation of every part of the drawing with the relations of each part to the whole object. It is always best to adopt some uniform way of making drawings and keeping notes. The system outlined below will be found very useful and convenient for examination.

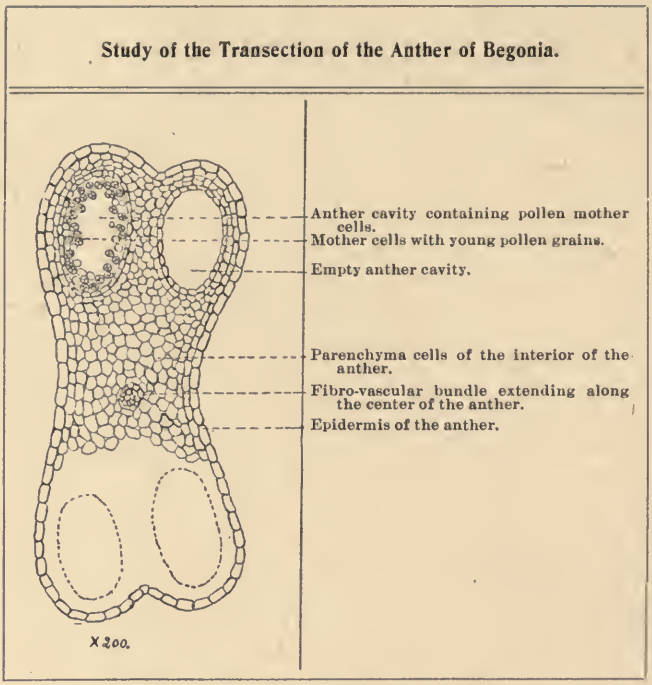

Fig. 5. Sample page of a drawing book.

The notes and explanations which accompany the drawings may be kept on separate sheets or pages in the drawing book.

Preservation of Slides.

To the person who makes a collection of microscopic slides, it becomes a matter of no little importance as to how they should be arranged in order that they may be best preserved and at the same time available for instant reference. Many kinds of cabinets may be purchased of dealers, but the large ones are quite expensive and beyond the means of many. A very inexpensive way to preserve the preparations is in mailing boxes, which hold 25 slides. These can be filed away but should be kept on end to prevent displacement of the mount. After a time, however, the number of boxes accumulates so as to become burdensome; and furthermore, it is better for slides to be supported from below on each side of the

cover glass, rather than at the ends. A servicable and convenient cabinet can be made by removing the drawers of an empty spool-case and making a door for the front. The case can then be filled with drawers suitable for holding the slides. To make these cut a board, one-half inch thick and as wide as the case is deep, into lengths one inch shorter than the width of the case. With the aid of a buzz-saw and chisel, the boards can be made so that a section of it will appear as the figure:

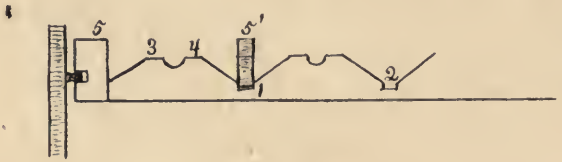

Fig. 6. Diagram showing the construction of a drawer for a slide case.

The grooves are to be cut across the grain of the wood. Cut thin strips and glue them in position in the grooves 1 and 2. The distance between these points should be $3\frac{1}{8}$ in. The slides rest on the surfaces 3 and 4, and are separated by little partitions, about $1\frac{7}{8}$ in. apart, glued into a groove made across the board with the saw before the partitions 5, 5′ were placed in position. The little partitions must necessarily be short, about $2\frac{1}{2}$ c.m. long in order that they may not come in contact with the pieces 5 and 5′. To remove the slide from the drawer, it is only necessary to press down on one end when the other will be raised and can readily be grasped.

Such a cabinet can be easily made by a carpenter in a few days. Much of the work can be done by machinery. The original cost of the spool case should not be over $3.00. Double spaces can be left in the drawers for the large sizes of slides and appartments arranged in any desired way. A piece $\frac{1}{2}$ inch thick fastened to the end of the drawer will prevent its warping and at the same time serve for a groove on which the drawer can slide.

The number of the first and last slide in each drawer can be fastened on a strip, to the front surface.

To catalogue the sections obtain cards $7\frac{1}{2}$x$12\frac{1}{2}$c.m. of good

bristol board. Each slide should have a corresponding card and on it the following data should be given:

1. Number of preparation.
2. Name of object, from what taken, and locality.
3. Name of preparer and date of mounting.
4. Object of preparation.
5. Method of mounting, stain, mounting medium, etc.
6. Reference to figures, books, and papers.
7. Remarks.

Sample Card.

No. 1.

TRANSECTION OF LEAF.

Oct. 29, '93. J. Smith, Preparer.

From Begonia Sanguinea. Green house plant.

Shows general structure of leaf, stomates, guard cells, etc.

Hardened with alcohol, infiltrated with collodion, stained with haematoxylin, and mounted in balsam.

Strasburger's Pract. Bot. p. 162.

This section is quite thin and should be studied with the high power, etc.

The cards should be arranged alphabetically according to subject and can be kept on edge in a box of convenient size, with the topics separated by tin or card board on which the letters of the alphabet are pasted. This arrangement places at hand for instant reference the whole collection of slides, and enables one to easily find any particular section, while it furnishes a record of valuable data with each preparation.

When any slide is permanently removed or destroyed, the card can be taken from its place and the set suffers no injury.

APPARATUS NEEDED.

Certain apparatus not supplied by the laboratory will be needed by each student in his work, while many things not actually needed will be found very useful and even quite indispensable for a thorough course.

The laboratory should be supplied with good compound and simple microscopes. The former should have a magnification of from 75–600 diameters, and the latter about 20.

As regards the most suitable microscope stand, there need be no discussion. The stands of any reliable maker can be used. It is important that the working parts be all accurately adjusted, the pillar provided with a joint for inclination, and the instrument be firm and substantial. The Continental stands of American manufacture are especially to be recommended, as they are quite compact and can be fitted with the various sub-stage parts with but little alteration. The instrument should be furnished with a nose-piece for the objectives and an ocular micrometer for measurements. For the simple microscope, the ordinary tripod magnifier answers the purpose very well and is useful in teasing material with needles under a low magnification. The laboratory should be provided with some form of a sliding microtome. Well microtomes can be used for many things, but for cutting serial sections or material that has been infiltrated with collodion, a sliding microtome is very desirable. The patterns of several good makers are on the market and can be secured of regular dealers. The large microtomes of Bausch and Lomb, or Reichart are especially to be recommended. They cost forty or fifty dollars but the advantages in their use will well repay the expense. The small student's hand microtome of

Bausch & Lomb, is very convenient, and, in case the large sliding one can not be secured, it will be found serviceable for almost all kinds of sectioning. Its cost does not exceed ten dollars.

Reagents, Etc.

The following is a list of the more general reagents used in the laboratory:

Hydrochloric, Nitric, Sulphuric, Acetic, Osmic, Chromic, Picric, and Carbolic acids.

Caustic Potash, Sugar, Potassic Iodide, Iodine, Chlor-iodide of Zinc, Glycerin, Schweizer's Reagent, Glycerin Jelly, Balsam, Shellac for sealing cover glasses, Turpentine, Chloroform, Ether, Gun-cotton, Xylol, Paraffine, Clove Oil, Clearer.

Haematoxylin (cryst.), Eosin, Carmine, Magnenta, and various Aniline stains.

Alcohol and distilled water should be provided in quantities for all the general manipulations in which they are required.

The students should provide themselves with glass slips with ground edges, 3x1 inches, and cover-glasses of assorted sizes. $\frac{3}{4}$ and $\frac{7}{8}$ in. circles are most frequently needed, but serial sections will require 1 inch square and a few 25x50 c. m.

Adhesive labels, 1 inch square, will also be needed for labeling the slides. For storing the mounted preparations, mailing boxes holding 25 slips are most convenient for student's use. Two camel's hair brushes will be needed one, for handling the sections, and the other for brushing dust from lenses, covers, etc.

The drawing material required should be a note book of unruled drawing paper or separate sheets cut the proper size can be used. "Ledger Linen" is to be especially recommended for this purpose. The sheets with notes and drawings can be fastened in a cover of manilla paper by boring two holes through the backs and fastening with a string.

Two kinds of drawing pencils are needed. If ink drawings are to be made, India ink and a fine pointed pen ("crow quill" or Mapping pen No. 291) should be provided.

Dissecting needles can be made by grasping a strong fine pointed needle between a pair of pliers and forcing the head into the end of a pine stick or a straight twig with a small pith.

It is very desirable that the laboratory be provided with an Abbe camera lucida and some high power objectives; e. g., a 1-10 or 1-12 oil immersion. A substage condenser is very desirable when such high magnification is to be used.

It is necessary that each student be provided with a good section knife or razor. One of the latter ground flat on one side and concave on the other is preferable. The edge should be kept very sharp and keen, otherwise the sections will be uneven and torn. A suitable hone and strap are needed in the laboratory for keeping the edge in order. Unless the students can be carefully taught how to sharpen a knife, it should be taken to some one familiar with honing razors. For cutting sections of lignified or cutinized material, an old razor should be used, as the edge will almost invariably be injured. Various pieces of apparatus not mentioned will be found very useful and materially aid in careful and thorough work, yet much can be accomplished without any very extensive expenditure of money for equipments.

CARE OF APPARATUS.

It is very desirable that the student should have had some previous training in microscopical manipulation, but to those who have had no such opportunity some few explanations and suggestions are necessary before they can work to advantage with so delicate an instrument as the compound microscope. Access should be had to some of the excellent books on microscopical technique mentioned elsewhere in this manual.

With reference to the care of the microscope it is especially important that the optical parts be kept free from dust or dirt, and in any case where the lenses come in contact with anything that would soil them they should be cleaned at once. The Japanese bibulous paper recommended for this purpose can be secured of G. S. Woolman, 116 Fulton street, New York. After cleaning a lens, the soiled paper should be thrown away or it may be used for removing liquids from any part of the instrument. Since the glass from which the lenses are made is quite soft, it should never be subjected to any hard rubbing, as its surface would be injured by scratching. Never touch any of the glasses with the fingers or allow the objective to come in contact with reagents or stains that may be on the table of the microscope. If balsam or shellac should get on the face of the lens, wet it with alcohol and remove *at once* with a linen cloth as the alcohol would soon injure the mounting. Glycerin and glycerin jelly can be removed with water.

Many of the operations in testing for different vegetable or mineral substances must be performed on the stage of the microscope, where the action of the agent can be determined. In this case, much care must be exercised in order that the

instrument may not be injured during the operation. Where a stain or a liquid of any sort is to be applied, place a small drop of it on the slide in contact with the cover. Then hold a bit of blotting paper on the opposite side in contact with the liquid, and the reagent will be drawn through under the cover glass where its action can be observed through the microscope. In case of the action of the acids that may cause effervescence, see that none of the particles of acid fly against the objective or stage of the instrument. Do not let any liquid come in contact with the microscope.

Always keep the stand free from dust, and when necessary to wipe any part of it rub with the grain of the finish to prevent scratching.

When necessary to lubricate any of the working parts, use a little soft tallow and wipe the surface slightly to remove any surplus oil.

If any of the parts become loosened by wear, see that the tightening screws are turned.

In inclining the body of the microscope, grasp the upper portion of the pillar and never allow any strain to come on the adjustments of the instrument.

In screwing the objectives in place, use both hands, holding the front of the objective between the first and second fingers of one hand, with the other turn it in place by grasping the milled head on the back of the objective. In removing it, reverse the operation.

To remove the ocular or change the length of the draw tube always grasp the course adjustment with one hand to prevent the objective from being forced down upon the stage.

Keep the lenses in a place where they will not be exposed to sudden changes of temperature, as the expansion or contraction of glass or metal might crack them.

In getting the object in focus, run the objective down until it is nearly in contact with the cover glass. Then look into the microscope and raise the objective by means of the course adjustment, as the image appears in focus, use the fine adjustment for further study. *Always focus up, never down.*

CARE OF THE EYES.

Some trouble may at first be experienced by those unaccustomed to the use of the microscope, but this will shortly be overcome if due care is exercised. Always work with both eyes open and divide the labor between the two. At first, it may be a little troublesome to see the objects in the microscope distinctly with both eyes open, but by a little perseverance this can be overcome and the objects outside will not interfere.

A convenient form of an eye shade can be made by covering a piece of card board, 10x18c.m., with black cloth. Make a hole in the card midway between the ends and 2c.m. from one side. Put the shield over the ocular, letting it rest on the collar of the draw tube. A rubber band fastened to the card will keep it in place. (Fig. 7.)

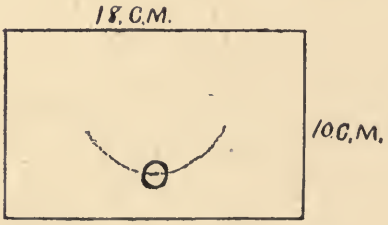

Fig. 7. Diagram of an eye shade.

This shield will cut off the light from the eye not in use, and be of material assistance.

Do not use the microscope after the eyes become tired. The fatigue which is troublesome at first will wear away in a short time, and one will soon be able to work for hours with the instrument without difficulty.

It is the common experience with microscopists that the eyes improve with use and are permanently benefited, the same as by judicious exercise of other portions of the body.

The light suitable for microscopical work should be strong enough to enable the object to be clearly seen, yet not so bright as to dazzle the eyes. *Always avoid direct sunlight.* The light from a north window is to be preferred, especially if the sky is clear or covered only with white clouds. Microscopic work can be carried on in the evening by lamp, gas, or electric light. Lamp and gas light are not very desirable and usually give unsatisfactory results.

The use of electric light is very highly recommended, provided a ground glass shade be fitted to the incandescent lamp. This gives a uniform, modified light and proves to be very satisfactory.

MANIPULATION OF APPARATUS.

Much has been written upon general microscopical technique, and some excellent books are easily procured, but it may not be amiss to call the attention of those who are beginning the work with the microscope to a few important precautions and directions that must be observed, and also to offer some few suggestions which may aid in manipulation.

Access should be had to Prof. S. H. Gage's "Microscopical Methods" which has been freely drawn from in the preparation of the following exercises.

Interpreting Appearances.

Dirt or Cloudiness on the Lenses.—It is important that the lenses be free from dust or dirt, and any cloudiness seen in the field of the microscope should be removed at once. For removing particles of dirt or dust, a camel's hair brush can be used, but for wiping the lenses an old linen cloth or Japanese bibulous paper is to be recommended. To determine the location of the particles of dirt, look into the microscope and revolve the ocular in its place. If the dirt is on the lenses of the ocular, it will revolve in the field of the microscope. If it does not, it is on the objective.

Should the trouble be with the ocular, remove it from the microscope and wipe its lenses thoroughly. If desired the front lens can be unscrewed and cleaned without injury to the instrument. In replacing the ocular, if it fits tightly, observe the precautions given elsewhere to prevent the objective from being run down against the stage of the microscope. If the trouble is not remedied by cleaning the ocular, remove the objective and wipe the front lens being careful not to scratch it.

Do not take the objective apart for cleaning. Should any repairs need to be made on the inside of it, return to the maker for examination. For removing balsam or glycerin jelly, see directions elsewhere, (p 56.) Having secured a clear field, i. e., the round area seen on looking into the microscope, some studies should be made in focusing. For this purpose prepare a glass slide as follows: place in the center of a glass slip a piece of paper about 1 m. m. square on which is the letter (a) in "diamond" type. Placé on this a drop of balsam and cover with a piece of glass about 1 c. m. square and 2 m. m. thick. On this piece of thick glass place the letter (b) very close to the letter (a), but not over it; add a drop of balsam and superimpose a piece of thick glass, the size of the one beneath. Repeat the operation with the letter (c) and place over this a thin cover-glass, the size of the thick glass below. After the balsam has hardened the mount can be sealed with shellac or asphalt. Place the slide under the microscope, using a $\frac{3}{4}$ or 1 inch objective. Focus on the letter (b), then turn the fine adjustment either way, and observe the effect. By continuing the experiment, the working of the adjustments of the instrument will soon be understood. A knowledge of this is absolutely necessary, since with higher powers, the direction in which the micrometer screw should be turned is of the utmost importance. It should also be known whether a certain part seen under the microscope is at the top or bottom of the object. It is only by much practice in this way, that the relations of structures are determined. Observe, also, that only one letter is in focus at once. This is because the *depth* of focus is not great. In general, this distance become less as the magnification is increased.

In order to examine thoroughly an object of much thickness, which is readily penetrated by the light, it must be studied in section; that is, focusing on one part and afterward on one above or below it.

The location of the parts with regard to their vertical position can be determined by the working of the micrometer screw. Such sections of the object as are in focus at any one time are known as "optical sections." Objects with irregular contour must be studied in this way.

Air Bubbles.

Take a clean slide and place in the center a few drops of mucilage or glycerin. Beat this with a knife until it has a frothy appearance due to the presence of numerous air bubbles. Cover with a cover-glass and focus on a small bubble. If the rays of light from the mirror pass perpendicularly through the slide, the bubble will have a light center and a uniform dark border. If the mirror is not central, the light spot will be at one side of the center. After taking off the sub-stage attachments, move the mirror bar until it is at an angle with the stage, and observe, when the light passes through the bubble, the direction from the center which the light spot has taken. It will be away from the side to which the mirror bar was moved.

Now beat some cedar oil or oil of cloves, and repeat the experiment. Observe the direction taken by the light spot in the oil globule. It will be contrary to that taken by the one in the air bubble, or to the side on which the mirror bar was swung.

It is interesting to mix the mucilage with the oil and find an oil globule and air bubble side by side. Then study the effect of oblique light to identify the bubble or globule.

It is advisable that the student familiarize himself with the more common objects that he will meet, perhaps, as foreign bodies in his studies. Mounts should therefore be made on a slide dry or in a drop of water of such objects as spores, dust, cotton and woolen fibers, hair, cork, etc., and studies made of them with the high and low powers.

MAGNIFICATION.

Any student working with a microscope should be able to determine the magnification of his instrument together with the ocular micrometer ratio.

Determination of Magnification by the Use of Wollaston's Camera Lucida.

Arrange the stage micrometer in position and focus on it until the lines are clearly visible. Place the camera lucida on the ocular and tilt the body of the microscope until the tube becomes horizontal. Then raise the base of the instrument upon blocks, until the distance from the ocular to the table is 25 c. m. Change the mirror until the light is reflected through the tube of the microscope, after which modify the quantity of light falling on the white paper, which should be placed on the table beneath the ocular, until, by looking through the camera lucida toward the table, the lines of the stage micrometer will be seen on the paper below. Mark off with a pencil the distance between the lines of the stage micrometer, as reflected on the paper, and measure with a rule. To determine the magnification, divide the size of the image by the size of the object magnified, and the quotient will be the magnification of the instrument, under the one condition. If the distance between the lines on the micrometer is 1-10 m. m., and on the image 50 m. m., then the magnification of the instrument in that condition is $50 \div 1\text{-}10 = 500$.

Determine the magnification with the several combinations of oculars and objectives.

The magnification can also be determined by the eye-piece and stage micrometers.

In the measurement of most objects the former micrometer is used. This is a scale ruled on glass and placed in a slit in the ocular, or inside, by unscrewing the upper lens of the combination.

To Determine the Ocular Micrometer Ratio.

Place the ocular micrometer in position in the slit in the eye-piece, and move the eye lens up or down, until the lines on the glass are distinct. Now place in position the stage micrometer, and the lines on it will appear below under those of the eye-piece micrometer. Move the two scales until the lines of each are parallel with the other. Measure with the scale of the eye-piece micrometer, the distance between the lines of the stage micrometer. The ocular micrometer ratio is obtained by dividing the number of spaces on the eye-piece micrometer, required to cover a space on the stage micrometer, by the value of the divisions of the latter. For example, suppose the markings of the stage micrometer were 1-100 of a m.m., and the number of spaces of the eye-piece micrometer required to cover one space in the former was 5, then, the ocular micrometer ratio would be $5 \div 1\ 100 = 500$, i. e., the ratio is 500. The value of each division of the ocular micrometer is 1-500 with the above conditions in the microscope. The ratio with the several objectives should be ascertained.

The magnification of an object can be determined, by dividing the size of the image, as measured by the eye-piece micrometer, by the ocular micrometer ratio. For example, suppose the dimension of the image of a cell to be 5 divisions of the eye-piece micrometer, and the ocular micrometer ratio is 300, then the size of the cell is $5 \div 300 = .0166+$m.m. The size of an object can likewise be determined by measuring the size of the image, under the conditions described for determining the magnification of the instrument with the camera lucida, and then dividing the size of the image by the magnification of the instrument. Suppose the size of the image is 5 m.m. on the paper, and the magnification of the instrument is 300 diameters, then the real size of the object is $5 \div 300 = .0166+$m.m.

The unit of micrometry as universally used is the 1-1000 of a m.m. This was suggested by Harting in 1859 and called, in 1869, by Listing, the *micron*. It is designated by the Greek letter μ. The magnification of an object is then always to be given in mi-

crons, and the reduction is simply made by multiplying the actual size of the object by 1-1000.

In all measurements with the microscope, the draw tube should be pulled out, until the whole tube of the microscope is of a certain length. This distance is known as the "tube length" and varies in microscopes of different makers, as do also the points between which the measurement is made. See Prof. S. H. Gage's Microscopical Methods, p. 10.

In order that the student may become familiar with the working of the microscope he should carefully go through the following exercises:

1. Putting the ocular and objective in position. p. 57.

2. Lighting the field of the microscope, with direct and oblique light. p. 62.

3. Determine the relative position of optical sections, and the manipulation of the fine and course adjustments. p. 61.

4. Study of air bubbles and oil globules with reference to the identifying of each by their appearance, under direct and oblique illumination. p. 62.

5. Study of currents in liquids and their direction upon inclination of the stage of the microscope. These currents can be produced by grinding upon a slide, with a knife, a little carmine in water, and covering with a cover glass.

6. Determine the magnification of the instrument with the various combination of lenses. p. 63–64.

7. Dirt or cloudiness on the lenses. p. 60. Smear the objective with glycerin and study the appearance. Remove the glycerin with water.

8. Mount various objects (hair, cotton and woolen fabrics, bits of wood, paper, thread, etc.), under a cover-glass in water, and study with the high and low power. In this way one will become familiar with the adjustments of the instrument and the appearance of the more common "foreign bodies" that might be found in ordinary preparations.

Part Second.

LABORATORY DIRECTIONS.

DIVISIONS OF THE SUBJECT:

A. Living Cells, (with Protoplasm and Chlorophyll.)
B. Contents of Cells, (the secondary products.)
C. Elementary Tissues.
D. The Primary Meristem.
E. The Systems of Tissues.
F. The Thickening of Stems, etc., (secondary growth.)

A. THE STUDY OF LIVING CELLS.

For convenience of study, cells may be classified as to manner of association as follows:

I. Those which live *separate from one another.* Examples, Unicellular Fungi and Algae, Pollen-grains, Spores, etc.

II. Those living in *Colonies,* i. e., joined temporarily, but which are able to perform all their normal functions if isolated; example, Spirogyra, (known as "Frog-Spawn," "Water-Carpet," "Pond-Scum," etc.)

III. Those which live *permanently joined* to other cells and which cannot ordinarily perform their normal functions if isolated; (a) they may *not* form *Tissue*; example, *Nitella*; (b) they may form *Tissue;* example, *Roots, Stems, Leaves of Flowering Plants.*

CASE I.

Isolated Cells Containing Protoplasm.

Illustration: *Protococcus viridis,* Ag. (green slime.)

The plant can be found in *damp places,* growing on the bark of trees, or in the corner of buildings on the brick, or stones of the foundation. In fact so general is the plant distributed that no one need have any difficulty in getting material in good condition for study.

PREPARATION FIRST: With a knife remove some of the material from the substratum and mount in water. Place under the high power of the microscope and OBSERVE:—1. The *unicellular plants,* often associated in *groups.*

2. Their *size, shape,* and *general appearance.*

3. The *thin colorless cell wall* surrounding a central granular mass of protoplasm.

4. *Chlorophyll granules* distributed through the protoplasm. These are the centers of the *vital processes* in the cell, and in sunlight by the decomposition of plant food form starch, protoplasm, etc.

5. The *nucleus.*

Stain the preparation with iodine and observe the effect on the wall, and protoplasmic contents. (See p. 7.)

Prepare another slide and stain with a fresh solution of *chlor-iodide of zinc*, to observe the effect of the *thin cellulose cell wall.* By pressing on the cover glass, the cell contents can be forced out and the cell wall be made more visible. (P. 11.)

Examine several specimens to observe the various stages in the division of the plants into groups of individuals.

Many of the small plants will be seen moving about through the water. This is due to the presence of *cilia* which, by their rapid movements, propel the individual. Parker's Biology, p. 23; Strasburger, p. 214; Vines' Text Book, p. 236; Campbell's Structural Botany, p. 22; Bibliography of the Literature on the Plant Cell, by Dr. A. Zimmermann; Botanisches Centralblatt, Beihefte, 1894.

Further Illustration: POLLEN-GRAINS and their MOTHER-CELLS, from Begonia sp——.

PREPARATION FIRST: For the MOTHER-CELLS of POLLEN. Lay out a perfectly clean glass slide and cover-glass, placing a few drops of distilled water on the former. Select a young *staminate flower bud* (less than half grown). Moisten the razor edge and make thin sections across the upper part of the flower buds, and the tips of the contained anthers. From these sections select the thinnest, especially those of the anthers and remove by means of a camel's hair brush to the slide. Examine these sections with a tripod lens or a dissecting microscope, removing the thicker anther sections and fragments of the perianth. Cover the sections with the cover-glass. The latter should be taken up with the forceps, one edge placed in the water, and the glass lowered, not too suddenly, but so as to allow the water to run along its lower surface without enclosing any air bubbles. Examine first with the low power objective ($\frac{3}{4}$ in.),

then with the higher (1-6 in.), using the C eye-piece and draw-tube when necessary.

Observe: 1.—The outline of the sections of the *anthers;* oblong or quadrate, usually with rounded angles.

2. The *cavities* near the angles;—later, these coalesce in pairs, thus forming the two "anther-cells," or pollen-cavities.

3. The *epidermal layer* of cells, the layer just beneath, and the irregularly placed cells of the interior of the anther.

4. *Isolated rounded cells* in the cavities or floating free in the water. These are the *mother-cells* of the pollens.

(a)—The very thin *cell wall*, its smooth surface, etc., has it perceptible thickness?

(b)—The *protoplasm* of the interior, its characteristics.

(c)—The *nucleus*, its appearance.

(d)—The *nucleolus*.

Further Preparation: If (c) and (d) are not clearly demonstraied apply a drop of iodine solution to one side of the cover-glass, and place a piece of filter paper on the opposite side. This absorbs the iodine and water, while the former acts on the protoplasm as a staining agent. (The iodine may be removed by using water in the same way).

Observe the relative amount of color given to the *cell wall* and the *cell contents;* especially the effect on the *nucleus* after the mother-cells have remained some time in the stain. Also observe the increased distinctness of parts. From this study determine the constant effects of iodine on protoplasm and refer to page 7 for the use of this reagent in plant histology.

Measure the diameter of the mother-cells by the use of the camera lucida, or ocular micrometer, selecting those of extreme and also of average breadth.

If practical retain this slide in a moist condition until the next preparation has been examined. It is useless to keep slides of soft tissue containing protoplasm more than a few hours when mounted in water. A temporary moist chamber can be made by placing a small plate partly filled with water on a level surface and covering it with a bell jar. The mount may be laid across a small watch glass inside. *Sketch* transection of anther from $\frac{3}{4}$ objective and a mother-cell from 1-6 objective. (Fig. 8).

PREPARATION SECOND: For young POLLEN-GRAINS. (Fig. 9.) Take a staminate flower somewhat older than the first. (The exact stage will have to be obtained possibly after trying several buds.) Section and prepare as before. Stain with iodine, letting it run under the cover one-third of its diameter. Focus on that region bordering on the stained and unstained portions.

OBSERVE: 1. The *mother-cells* (of pollen grains), floating free in the water, and the exceeding thinness of the walls.

2. The *contents* of the mother-cells—*four small "pollen grains"*; or if only three appear focus carefully to ascertain the presence of a possible fourth.

3. *Form* of pollens; their *nucleus* and *protoplasmic contents*.

4. The readiness with which the protoplasm of the pollen becomes stained, even when the water medium appears scarcely tinged.

5. *Mother cell wall*, almost colorless, although the iodine must have passed through it. Why?

6. Is the place between the mother-cell wall and the contained pollens more tinged than the water medium?

Figures of developing pollen grains, Goebel, p. 362; Strasburger, p. 313; Sachs' Physiology, pp. 99, 100.

Draw one or two mother-cells with contained pollens.

PREPARATION THIRD. FOR MATURE POLLEN GRAINS. From the mature anther of an open flower jar out the pollen, letting it fall on a dry slide. Examine carefully with a microscope, and then apply water to the side of the cover-glass.

OBSERVE: 1. Appearance of the *pollen grains* when on the *dry* slide.

2. *Change of form* when the water is applied; the cause?

3. *The wall*, its relative thickness as compared with that of the mother cells.

Measure a large and small grain, the longer and shorter axis. Draw a grain in the dry condition, and one in the moist. Sachs' Botany, p. 15; Bessey, p. 23; Gray's Structural Bot., pp. 256-257; Bot. Centralbl., Beih., 1893, pp. 206-17, 321-54, 401-36.

Illustration Second: POLLEN-GRAINS from the Order *Malvaceae*, (either *Hibiscus* or *Abutilon*.)

PREPARATION FIRST. This should be similar to that of preparation third under Begonia.

OBSERVE: I. The short *processes*, sometimes lobed, covering the face of the grain.

II. If the water causes the grain to swell.

III. The *size*,—measuring with the micrometer.

PREPARATION SECOND: *Culture* of POLLEN GRAIN and the study of germinating POLLEN TUBES, *Tradescantia* or *Begonia*.

Sterilize a slide, cover-glass, and ring, for making a cell. This can be done by heating them for some time in an oven at about 128° C., or quicker, by passing them slowly through the flame of an alcohol lamp. Make a 10 per cent. sugar solution and boil it ten minutes. Then with a platinum wire, cooled after passing through an alcohol flame, place a small drop of sugar solution in the center of the sterilized cover-glass. Sprinkle on this a very few pollen grains and invert over the ring, which is placed in the center of the slide and held in position by a drop of water at its lower edge. The culture will then have the arrangement shown in Fig. 8. Remove the slide to a moist chamber and allow it to remain in a warm place for a few hours.

Fig. 8. Showing the method of cultivating pollen grains in a hanging drop. A. Glass slide. B. Glass ring. C. Hanging drop containing pollen grains. D. Cover-glass.

Examine the pollen grains after a few hours with a 1-6 objective *taking care not to break the cover-glass* by forcing the lens against it.

OBSERVE: 1. The outer and inner coat of the grain; the former is called the *extine*, the latter the *intine*.

2. *Pollen tubes* of varying lengths projecting from the several grains, usually but one tube from each grain (there may, however, be several).

3. The *thin wall* of the *pollen tube* continuous with the intine, and the *extine* ruptured by the germination.

4. The *granular contents* of the pollen tube consisting of *protoplasm* and *starch*, together with the *nucleus* of the grain, on

its passage to the end of the tube, from which it goes to perform the office of *fertilization* when in contact with the *oosphere* of the *embryo sac*. If the pollen grains of Malvaceae are used, observe the relation of the *pollen tubes* to the *processes* or *protuberences* on the grains.

The culture slide can be kept for several days, and the development of the pollen tubes observed at intervals. For figures of developing pollen tubes see: Bot. Gazette, 1886; Goebel, p. 365; Strasburger, pp. 304, 320; Goodale, p. 429.

CASE II.

Cells in Colonies, Joined Temporarily.

Showing CELLS in COLONIES, also PROTOPLASM, CHLOROPHYLL in SPIRAL BANDS, and PROGRESSIVE CELL DIVISION.

Illustration: *Spirogyra.* This genus of filamentous unbranched aquatic plants belong to the Conjugatae, a group of Thallophytes (See Sachs' Text Book of Botany, p. 25; Bessey's Botany, p. 232.) Spirogyras, when in the vegetative state, are bright green, and have a silky luster when taken from the water. They vary much in diameter of the filament in different species, but are seldom over .15 m. m. They frequently occur in fresh pools or slow flowing streams, and may be found in winter in pools that do not freeze. Most species pass into a reproductive stage in early summer. But few species are known to conjugate in the winter.

PREPARATION FIRST: Place a few of the filaments on a slide in water.

OBSERVE: 1. *Cells* placed end to end.

2. The *septa*, or *transverse partitions*, plain in some filaments; possibly in others a *box-like area* will be observed at the septum.

3. The *spiral band* or *bands*, of bright green color, part chlorophyll pigment and part protoplasm. Ascertain if possible the number of bands. To do this, count the number that cross a thread between the two points where it touches the opposite sides of the cell, and this number plus one, will be the number of bands in the cell.

4. The *irregular clearly cut margin of the bands*, and the protoplasmic ridge traversing the middle of each. If these are not well defined the cells are not in a healthy condition.

5. Globose *masses* or *corpuscles*, in the band at intervals. These corpuscles are apparently centers of protoplasm, where starch grains are produced, under proper conditions. The corpuscles and the connecting protoplasmic ridge are regarded by Schmitz as one continuous "chloro-plastid."

6. The transparent *roundish area* at the middle of each corpuscle,—the *pyrenoid* of *Schmitz*.

7. The *cell nucleus* if present; its form. It may be more clearly brought out by the application of a little dilute iodine.

8. If *protoplasm* can be detected in the cell, except in the chlorophyll bands.

PREPARATION SECOND:—Stain a preparation with iodine, by placing a drop at the edge of the cover glass.

OBSERVE: 1. The effect on the *corpuscles*, *nucleus* and *protoplasm* in various parts of the cell.

2. That the *cell wall* remains unstained or slightly colored although the iodine must have passed through it.

3. The slow action of the iodine on the *chlorophyll bands*. The cause of this slowness.

PREPARATION THIRD:—Stain as before, using *strong* iodine instead of the dilute solution.

OBSERVE: 1. *Contraction* of the whole cell contents in some cases. It is a *coagulation* of the protoplasm, the water being expelled by the iodine.

2. The *color* given to the different parts.

For the study of cell division, keep the plants in a very cool place over night and bring in to the warm laboratory in the morning. The division will begin in a short time. If the plants are kept constantly in a warm place, the division will usually take place at night. In specimens treated as above directed,

OBSERVE: 1. The *ridge* of *cellulose* being pushed inward from the side walls in some cells. This is a *septum* in active formation, (by progressive cell division).

2. The *chlorophyll*, etc., continuing through the *aperture* of the partly formed septum. Sketch the above appearance.

Describe the plant and all the phenomena observed, carefully and concisely, including the effects of the staining agents.

Sachs' Botany, p. 16; Vines Text Book, p. 118; Parker's Biology, p. 192; Goebel, p. 49; Strasburger, p. 246; McAlpine's Charts, pl. iv; 19th Smithsonian Contributions, pl. 14 and 15.

For method of making permanent mounts of Spirogyra see p. 38.

CASE III.

This includes by far the largest number of plants existing. They may be considered under two heads.

A. Those which do not form Tissue, of which, *Chara* and *Nitella* may be taken as a type. The same condition exists in stamen hairs, many trichomes, etc.

B. Those which form Tissue, as the higher *Cryptogamia* and the *Flowering Plants*, which will furnish the illustrations under the subsequent heading of *Tissues*.

Case III. (A.)

Illustration First: Stamen Hairs of *Tradescantia* showing protoplasm in motion. (Streaming movement.) (Fig. 10.)

Preparation. Remove from a perfectly fresh, newly opened flower a stamen with the attached *hairs* or *trichomes*. Place them in water taking particular care not to break or injure the trichomes.

Observe: 1. The *number* and *form* of the *trichome cells*.

2. The faint *pink color* of the *cell sap*, due not to colored granules, but to a *red pigment* held in solution.

3. The *nucleus*.

4. Slender *streams* of *moving protoplasm*. Trace their course.

5. Estimate their *rate of movement*.

For figures of the above see Strasburger, p. 29; Sedgwick and Wilson's Biology, p. 30; Bessey's, p. 12.

The *movement* of *protoplasm* can likewise be studied in the young plant hairs of *Cucurbitaceae*, in *Nitella*, or in *Vallisneria*. Any of the specimens can be mounted in water and treated the same as *Tradescantia*.

B. CELL CONTENTS.

The several illustrations following, represent the secondary products of the cell as distinguished from active protoplasm and its immediate derivaties, the chlorophyll body. The secondary products are found chiefly in the fundamental or cellular tissue, the characteristics of which will be observed. The solid forms of these products will be studied as follows:

1. **Starch Grains.**
2. **Crystals.**
3. **Protein granules.**
4. **Crystalloids.**
5. **Aleurone grains.**

Illustration First: Showing PARENCHYMA CELLS with STARCH. The TUBER of the POTATO. (*Solanum tuberosum.*) (Fig. 11.)

PREPARATION: Cut a fresh surface on a tuber, make several thin sections from 2–3 m. m. in breadth and as thin as possible. Mount in water.

OBSERVE: 1. *Form* of the *parenchyma cells* of the tuber.

2. *Ovoid* or *pyriform starch grains*, of various sizes, lying in and about the cells.

3. *Structure of grains;* some plainly showing a *hilum*, around which appear *rings* more or less *eccentric*. The rings are layers of greater or less density.

4. *Measure* some of the larger grains.

5. Run a drop of weak iodine under the cover and note the *blue color* resulting in the grain, the *characteristic reaction for starch*. Sketch a tuber cell with contained starch grains. Vines'

Text Book, p. 110; Strasburger, p. 11; Goodale, p. 49; Bessey, p. 54; Bot. Centralbl., LV. (1893) p. 157.

Illustration Second: Showing CRYSTALLOIDS. HYPODERMAL TISSUE of POTATO TUBER.

PREPARATION: Sections should be made below the cuticle, but very near the surface, and the parenchyma should contain but a few small starch grains.

OBSERVE: 1. The *rectangular cells* with cell *nuclei* if present.

2. *Crystalloids, cubical* in outline, surface plane, or sometimes eroded.

3. Upon the application of iodine, observe *yellowish tint* given to *crystalloids* indicating their *nitrogenous* nature.

4. After drawing off a considerable portion of the fluid under the cover-glass and subsequently applying a saturated solution of common salt (NaCl), observe the changes in, and final *dissolution of*, the crystalloids. *True crystals* are not thus acted upon. Strasburger, p. 25; Goodale, p. 47; Vines' Text Book, p. 111.

Illustration Third: ALEURONE AND STARCH. THE GARDEN PEA (seed of *Pisum sativum.*)

PREPARATION: Separate the cotyledons of the pea, and cut away a portion of one with a knife. Then with the heel of the razor make several small sections from the smooth surface. Mount in glycerin.

OBSERVE: 1. The *grains of starch;* their *form* and concentric lines of structure or *stratification.*

2. Their lines of *fissure.*

3. The minute grains in the cell with the starch, the *aleurone grains.*

4. The *cell structure* and *intercellular spaces.*

Draw, showing the above characters; then treat the section with strong iodine and observe its effect on the *aleurone.*

Goodale, p. 47; Vines' Text Book of Bot., p. 112; Strasburger, p. 18.

Illustration Fourth: STARCH, ALEURONE, and the STRUCTURE OF CEREAL GRAINS. Grain of WHEAT (*Triticum vulgare.*)

PREPARATION: By cross-section cut away one-third of the grain. Make several thin sections from this surface, which shall include only a small portion of the coats of the grain, and of the

white interior. The coats are better developed near the groove or sinus of the seed. Mount in glycerin or water. The sections can be more easily prepared by soaking the grains for a few hours in water.

OBSERVE: 1. The *form* of the *cells* of the interior of the grain.

2. The form of the *starch-grains* in these cells. The stratification is not often visible. It can be detected in some upon the application of dilute *Chromic Acid.* This is a useful agent in rendering the stratification of starch-grains, cell-walls, etc., more distinct.

3. The form of the *Aleurone-cells* (or "*gluten sacs*") inclosed by broad white walls, whose outlines may be traced even on their peripheral side. The Aleurone-cells lie next to the starch-bearing cells.

4. The minute yellowish grains of *Aleurone* filling these sacs.

5. The *Seed-coats* and the *Ovary-coats.*

(a) The *Inner seed coat*, (or *Secundine*) made up of two layers of white homogeneous cellulose, separated by an interrupted granular line or thin layer. The peripheral of these two layers is regarded as the true "Inner seed-coat."

(b) The *Outer seed-coat* (or *Primine*), usually a narrow *brown* band. In this lies the color of "red" and "white" wheat.

(c) The *Endocarp*, apparently marked at intervals by oblong transverse pits, pinkish from refracted light. The Endocarp consists of a layer of elongated, thick-walled cells, running transverse to the long axis of the grain, therefore parallel to this section. The oblong pits referred to are narrow, nearly-closed passages through the party walls between these cells.

(d) and (e) The *Exocarp.* This is made up of several layers (usually two) of oblong, thick-walled cells running parallel to the long axis of the grain. The ends of these cells will therefore appear in the present section as oblong openings. When first mounted they are not always apparent. These thick-walled cells of the Endocarp, Exocarp, etc., are Sclerenchyma cells, and characteristic of the protective coverings of seeds.

It will be noticed that in the above illustrations the cells inclosing starch, etc., belong to the *Cellular Tissue.*

Further Preparation: The coats above named and their component cells will be more clearly understood if two or three thin, *tangential sections* be made, i. e., sections parallel to a plane tangent to the surface of the grain, and extending only to the starch-cells. Trace here the *Aleurone cells*, the *Outer seed-coat* (*Primine*), the *Endocarp*, the *Exocarp*. If iodine be applied to either the transection or the tangential section, and be followed by strong sulphuric acid, the inner seed-coat, the Endocarp and Exocarp turn a deep blue (the reaction for cellulose), then dissolve, leaving the brown *Outer* seed-coat entire, thus indicating the *corky* nature of the latter.

The above study should be made with care as all cereals show the same relative arrangement of seed-coats, aleurone-cells, and starch-bearing cells. It will make more clearly understood some of the processes of "high milling," by which the nitrogenous compounds (the Aleurone grains), are separated and saved from the bran. The ovary and seed-coats form the bran proper; the aleurone-sacs contain most of the nitrogenous compounds or albuminoids, and the phosphates; the interior cells contain the starch, which makes up the bulk of the "white" flour.

For figures illustrating the structure of the coats of the wheat-grain, see Report of the U. S. Com., at Vienna Ex., 1873, vol. 11, p. 4, on Art on Vienna Bread; Goodale, p. 47, 181; Strasburger, p. 19; Vine's Text Book of Bot., p. 112; Landois and Stirling, Human Phys., p. 444.

Illustration Fifth: Starch. Grain of Indian Corn. (*Zea Mays.*)

The semi-transparent portion of the dry grain should be used, and the sections made very thin and necessarily minute. The cells of the seed should be observed, but particularly the form of the *starch-grains* and the peculiar stellate cavities at the center of some when the section is first mounted in water. For figures, see Sachs' Botany, p. 61; Goodale, p. 181; Bessey, p. 55; Strasburger, p. 12; Vines Text Book of Bot., p. 112.

Illustration Sixth: Starch in Compound Granules. Grain of Oats. (*Avena sativa.*)

Remove the palets from the grain or seed, and mount the sections of the later in *glycerin* to prevent the disorganization of the compound granules. Observe the reticulated appearance of the latter, due to the lines of union of simple grains of starch; observe the form of the compound granules. (For figures see Goodale's Botany, p. 49, p. 181; Strasburger, pp. 11 and 12.)

With a little care the coats of the above grains may be sectioned for examination at the same time as the starch and compared with those of the wheat. In Fig. 139 (Goodale) the layer (a) is that of the *palet*, and should not be represented in connection with the true coats of the grain itself. Sections of seeds may be permanently mounted by removing the water and adding a drop of glycerin jelly. Seal when hard. Strasburger, pp. 11 and 12.

Forms of Plant Crystals { 1. Raphides—needle-shaped. 2. Crystal Prisms—prismatic. 3. Sphæraphides—compound, spherical.

Illustration Seventh: CRYSTALS.

Illustration for form 2. The outer dried lamina of an ONION bulb, (*Allium Cepa.*)

PREPARATION: Split a thick brown lamina parallel to the surface; this may be done by twisting two parts in different directions with the hands. The thin margin of the rent including the cells on the convex surface may be mounted in water.

OBSERVE: 1. The form of the *cells* in which the crystals lie.

2. The various forms of single crystals, viz: *Prisms* often with modified angles, also *hexagons* and *pyramids* if present.

3. The number of *principal faces* to the prisms.

4. The *double crystals* in the form of an oblique cross; *triple crystals* if present. Do the axes of such crystals pass through one another?

TESTS FOR CRYSTALS:

The greater number of plant-crystals are *Calcic Oxalate.*

A limited number are *Calcic Carbonate.*

Crystals of other salts of lime occur but rarely.

Calcic Oxalate is *not* acted on by Acetic Acid.

Calcic Oxalate is dissolved by Hydrochloric Acid *without effervescence.*

Calcic Carbonate is dissolved by both acids *with effervescence.*

As the composition of the crystals is uncertain, test with

Acetic Acid first. If it has no effect, draw it off, and apply Hydrochloric Acid. Make sure the acids reach the crystals and particularly observe the effect on any which may have floated out of the cells.

Tests for Calcic Phosphate and Calcic Sulphate are given in Poulsen and Trelease's Botan. Micro-Chemistry, p. 96; Zimmermann's Microtechnique, pp. 64, 62.

The Study of Group I. *Raphides* will be reserved until later in the outline, in connection with an examination of growing roots, in which these crystals are found in abundance near the tip.

Illustration Eighth: Cystoliths. These are the concretions composed of cellulose and a salt of lime, and are found chiefly in the epidermal regions of certain *Urticaceae.*

Illustration: Leaf of Ficus Elastica (one of the "*Caoutchouc Trees.*)

Preparation: Select a piece from the upper part of a leaf and mount the thin transection in water. See p. 16.

Observe: 1. The upper *Epidermis*, a layer of small colorless cells on the upper surface of the leaf.

2. The *Hypodermal layer* of larger, also colorless cells just beneath the upper Epidermis.

3. Larger cells at intervals in the hypodermal layer containing the concretions—the *Cystoliths*, attached to the peripheral side of the cell by a stalk.

Apply to this the preceding test for crystals, and demonstrate what salt of lime is present.

After the test has been completed, and the acid washed out, apply strong iodine, and observe the skeleton of cellulose remaining in the Cystolith. DeBary, p. 44, 104–105; Bessey, p. 11; Vines' Text Book of Bot., p. 108; Zimmermann's Microtechnique, p. 61.

Inulin.

Illustration Ninth: A substance of the same chemical composition as starch and found dissolved fn the cell sap of many plants: *Dahlia root*, or *Jerusalem Artichoke.*

Preparation: Make thin transections of a piece of *Dahlia* root that has been left for some days in strong alcohol or glycerin.

OBSERVE: 1. *Sphaerocrystals* of various sizes, adhering to the cell walls.

The *radial* and *concentric stratification* of the crystal; seen more clearly by the application of *nitric acid.*

Remove the alcohol by water and note that after a little time all of the crystals have been dissolved. Sulphuric acid also dissolves the crystals. Zimmermann's Bot. Microtechnique, p. 78; Vines Text Book of Bot., p. 114; Strasburger, p. 51; Comptes Rendus cxvi (1893), pp. 514–17.

C. ELEMENTARY TISSUES.

TABLE OF TISSUE FORMS, OR ELEMENTARY TISSUES.

I. Parenchyma Tissue.	IV. Fibrous Tissue.
II. Collenchyma Tissue.	V. Tracheary Tissue.
III. Sclerenchyma Tissue.	VI. Sieve Tissue.

VII. Laticiferous Tissue.

I. Parenchyma Tissue. Tissue of the Fundamental System, forming the "*ground work*" of plants; with cell walls of varying thickness, and although of diverse forms yet never fibrous, as distinguished from Prosenchyma.

The more ordinary forms to be considered are:

1. *Isodiametric.*
2. *Stellate.*
3. *Ellipsoidal.*
4. *Irregular.*
5. *Epidermal.*
6. *Suberous.*

Illustration: Isodiametric cells from Geranium Stems (*Pelargonium inquinans*), Pith of most Stems.

Preparation First: A thin transection of the stem can be made free hand as directed on p. 16 and mounted in water or glycerin.

Observe: 1. In the center of the section, *thin walled isodiametric cells* of varying size.

2. *Nuclei* if present.

3. *Contents* of *parenchyma cells* in various parts of the section.

4. The *irregular spaces* between the cells,—*Inter-cellular spaces.* (Fig. 12.)

5. By careful focusing near the outer edge of the section

where the cell walls are somewhat thickened, the *primary partition* between contiguous cells: *The middle lamella* or *inter-cellular substance*, as it is sometimes called. This lamella is of much the same chemical composition as the remaining cell walls of the tissue, and undergoes modification with it.

Preparation Second: Ellipsoidal cells, from the root of any herbaceous plant;—those of the *Hyacinth* grown in water are easily obtainable.

For this study a longisection of a root should be prepared in the manner directed for the previous study, or better, after the directions on p. 21.

Observe: 1. Thin walled *ellipsoid cells*, beneath the epidermal layer, making up the main body of the root—the *cortex*,—and extending to the *central cylinder*.

2. The cells toward the apex of the root are more isodiametric.

3. The *cell contents*, together with the general characters of the cortical tissue. Bastin's Bot., p. 152.

Preparation Third: Epidermal and Irregular Parenchyma cells from the leaf of Scarlet Geranium.

Remove a portion of the epidermis of the lower surface of the leaf. This may be done by raising it at a cut margin and stripping it back. It will come away as a transparent membrane. Mount in water with the external surface of the epidermis uppermost.

Observe: 1. The *irregular* outline of the colorless *epidermal cells*.

2. *Stomates*, with elliptical openings, at intervals.

3. *Trichomes* in various stages growing from the *epidermis*. The *elongated cylindrical cell* or cells composing them; the globose glands at the apex of some. (Fig. 25.) This illustrates the fact that cell walls, when not acted on from without, will develop with curved surfaces. Bastin's Bot., p. 156.

Preparaton Fourth: Make thin cross-sections of small portions of leaves, hardened and partly bleached, in 50 per cent. alcohol. In these the *chlorophyll bodies* retain their form and some of their color. The green tint imparted to the alcohol shows the readiness with which the *chlorophyll pigment* is extracted by its solvents. While sectioning, the razor and the leaf must be kept sup-

plied with alcohol to prevent access of air. Mount in alcohol or glycerin.

OBSERVE 1. *Epidermis* of the upper and lower sides of the leaf, appearing almost colorless as seen in section.

2. The thickened (cutinized) outside walls of the cells, which are in very close contact with each other.

3. The *palisade cells* (ellipsoidal), and the underlying layers of *irregular parenchyma cells* making up the *mesophyll* of the leaf. These cells form the main body of nearly all leaves. Note the large *irregular intercellular spaces* in connection with them, also their contents in the sections of fresh tissue.

4. *Stomates* in section over small *intercellular spaces.*

PREPARATION FIFTH: STELLATE PARENCHYMA from STEMS and PETIOLES of many aquatic plants.

Liliaceæ and *Pontederiaceæ* furnish excellent material for this study.

Make several thin transections from the stem or petiole of *Pontederia* and mount in water.

OBSERVE 1. The firm *regular epidermal parenchyma* with slightly cutinized walls.

2. The loose *isodiametric cells* making up the interior of the section.

3. Large *air-passages* occupying a considerable portion of the section.

4. Certain spaces filled with *stellate cells*, having their projecting ends in contact and forming a *sieve-like plate* across the cavity. (Fig 12.)

The function of these plates is probably to form a support for the cross running fibro-vascular bundles, and at the same time allow the free passage of air through the stem (Bot. Jahresbericht; I, 196.)

5. The large nuclei and granular protoplasmic contents.

PREPARATION SIXTH: *Suberous cells*, from the "CORK OAK," *Quercus Suber*, (commercial cork.)

Mount several very thin sections in water; mount others in alcohol on the same slide. *Suberin*, the peculiar substance of cork cells, repels water, and the comparison between the two preparations should be made as soon as possible.

OBSERVE: 1. If there is a larger amount of air imprisoned in one preparation, than in the other.

2. The *form* and *color* of the cells, their thin walls resembling the parenchyma previously studied. Apply concentrated *chromic acid* to the section and note the effect. Suberin will enable the cork to resist for a long time, (often many days and perhaps continuously), the action of the acid, while all other modifications of cellulose are readily dissolved by it.

The probable absence of cellulose from suberized walls, as presented by Eugene Gilson, is interesting to note in this connection, (La suberine et les cellules du liege. La Cellule, etc., p. p. Carony, T. 1890 p. 63.) See Zimmermann's Microtechnique, p. 148.

MODIFICATON OF CELL WALLS.

Those that produce changes in the *chemical composition* of the cell wall may be classified as follows:

1. *Mucilaginous Modification.*
2. *Lignification.*
3. *Cutinization.*
4. *Mineralization.*

Illustration First: Cell walls containing MUCILAGE. SEED COATS of FLAX or LINSEED.

Make a thin tangential section of the seed, and place for a few minutes in water.

OBSERVE: 1. The ready *absorption* of water, and consequent *thickening* of the cell wall.

2. Its *gelatinous consistence* in seeds that have been left for some time in water.

In many cases the modification goes so far as to convert the cell wall into a gum, soluble in water. Examples of this can be found in the Plum, Cherry, or Peach trees. Vines' Text Book of Botany, p. 107; Goodale, p. 34; Strasburger, pp. 99, 340.

Illustration Second: Cell walls containing LIGNIN. Fibrous tissue of the stem of any woody Phanerogams, or Vascular Cryptogams.

Make a thin section of a woody stem and mount in a few drops of cuprammonia. Note the walls are not dissolved, which would be the case, if they were *pure cellulose.* (P. 5.)

To a fresh section add iodine and sulphuric acid, or chlor-

iodide of zinc, and observe that the cell walls are *colored yellow* or *brown—the characteristic test for Lignin.* What would be the reaction with pure cellulose? Zimmermann's Microtechique, p. 143; Goodale, p. 36; Vines' Text Book of Bot., p. 107; Strasburger, pp. 59, 119.

Illustration Third: Cell walls containing CUTIN. Epidermis of most plants, and in many tissues where cell walls are to be strengthened, or protection secured.

Make thin transections of the leaf of *Pinus Sylvestris* or *Cycas*, and mount in water.

OBSERVE: 1. The small epidermal cells with outer walls much thickened, usually in layers by the formation of *cutin.* The cutin can be removed by leaving the tissue in strong *chromic acid* for some time. After this, wash the section with water and add chlor-iodide of zinc. Note the *blue color*, the reaction for *cellulose*, forming the greater per cent. of the inner part of the wall, while the outer part consists of nearly pure *cutin.* Vines' Text Book of Bot., p. 107; Goodale, p. 38: Strasburger, p. 58; DeBary, p. 78.

Illustration Fourth: Cell walls containing MINERALS, in CRYSTALLINE OR AMORPHOUS forms. (Stems of Cereals, and many Sedges.) The substance most frequently present is Silica. To test this, ignite the specimen in a platinum dish, treat with nitric acid, and again ignite. The *silica* remains behind, and often retains the microscopic form of the tissues. This is true with the stems of Equisetum (horsetails.)

Crystals of *Calcium salts* can be found in the cell walls of the bast tissues of the willow and many Gymnosperms. For further study of the subject, examine Vines' Text Book of Bot., p. 108; Goodale, p. 39: DeBary, p. 102.

Continuity of Protoplasm.

Illustration: Stem of *Aesculus* (common horse-chestnut.)

PREPARATION: By the use of a knife remove the outer dark colored and inner green bark (periderm), from a young growing stem, about 1 c.m. in diameter. With a sharp razor make thin tangential sections of the exposed whitish tissue (cortex), and place in a solution of iodine in potassic iodide until brown. Wash

thoroughly with water and add strong sulphuric acid. After a few minutes repeat the operation of washing.

The acid renders the cellulose cell-wall transparent, and the protoplasmic strands can be seen connecting the masses of contiguous cells. If this does not show clearly stain the preparation with aniline blue. The sections can be permanently mounted in a drop of glycerin jelly.

Strasburger, p. 371; Quart. Journ. Micros. Science, 1882, p, 365, 1883, p. 151; Bot. Gazette, 1889, p. 83.

II. Collenchyma Tissue.

This tissue is composed of *parenchyma like cells* with walls *thickened at the corners*, or *points of contact*, and usually *tapering* at the ends. They form cylinders of tissue beneath the epidermis in many herbaceous stems and petioles. The cells often contain chlorophyll and are sometimes capable of division.

Illustration: Collenchyma cells from a mature stem of the Begonia, or Geranium.

Preparation First: Transections of a stem 3 to 4 m.m. in diameter should be cut free hand, stained with haematoxylin, and mounted in glycerin jelly; or better, hardened in alcohol, infiltrated with collodion, sectioned, and mounted as directed on p. 21.

Observe: 1. Thin walled cells forming the epidermal tissue of the stem.

2. Inside of this a ring of *glistening thick walled cells* forming a zone about the stem.

3. The latter, the *collenchyma tissue* is composed of cells with walls comparatively thin along the lateral surfaces, but thickened at the angles. This tissue is very strong and contributes greatly to the strength of the stem.

Make a longitudinal section of the stem and examine the cells of this zone.

De Bary, p. 119; Strasburger, p. 106; Goodale, p. 65.

Endodermal Cells.

A modified form of thick walled parenchyma is found in the Endodermis, in most roots a single layer of cells, surrounding the fibro-vascular bundles of the central cylinder. (Figs. 17, 22.)

The walls of the cells are thickened and often folded at the points of contact between contiguous cells of the sheath. They are strongly *suberized*, and have a clear glistening appearance.

Illustration: Endodermis of the central cylinder in Hyacinth or corn root, grown in water.

Make a thin transection of an old root, and prepare as for the previous study.

Observe: 1. Near the center of the root surrounding the *fibro-vascular bundles* a single layer of cells thickened only at the points of contact, but the walls uniformly suberized, thus giving them a glistening appearance,—*the endodermis.* De Bary, p. 121; Vine's Text Book of Bot., p. 165; Strasburger, p. 136.

III Sclerenchyma Tissue.

The cells under this tissue form vary from the short *isodiametric* "*Stone cells,*" to *elongated fibres*, with *thick* walls, passing by gradations into *fibrous tissue* on the one hand, and into *cellular tissue* on the other, (refer to parenchyma). They will be here considered as distinct elements.

Illustration: (of "*Stone cells*), Fleshy roots of *Dahlia variabilis.*

Preparation First: Longitudinal sections should be made just beneath the brown epidermis. The zone containing the cells will be apparent by cutting or scraping off the epidermis with a knife, when the *sclerenchyma tissue* will be found as a hard gritty layer. Mount in water.

Observe: 1. The thin walled parenchyma, making up the main body of the fleshy root.

2. Groups of *Sclerenchyma cells* with very thick walls; the slender *canals*, often branched, running through the wall from the central cavity.

3. The meeting of the canals of adjacent cells.

4. The form of the *canal openings* as seen on the surface of the cells. (Fig. 13.)

Preparation Second: Sclerenchyma cells from the "Ivory nut," (*Phytelephas macrocarpa*) often found made into buttons, umbrella handles, etc. Some of the more common nuts may be used.

With an old razor or sharp knife, small sections of sufficient thinness can be obtained from the surface of the nut. These may be mounted in water for study, or in balsam for permanent preservation.

OBSERVE: 1. The tissue of the shell composed entirely of *sclerenchyma* cells.

2. The *narrow cavities* of the rectangular cells.

3. Very thick but clear cell walls; cell contents.

4. The *unbranched canals* passing through the walls at various places.

Enlargement of the canals at their place of union between contiguous cells. In many cases the septum between the two is not absorbed.

6. The peculiar branches of the canals at the end walls of the cells.

PREPARATION THIRD: SCLERENCHYMA CELLS from the underground stem of *Pteris aquilina*, (common brake). Place some of the underground stem between two pieces of cork, and fasten in the jaws of a microtome. With a strong razor kept wet with alcohol cut *thin* transections and mount in glycerin jelly for permanent preservation.

OBSERVE: 1. The *bands* of dark *reddish brown tissue*, extending across the section. These bands are composed of sclerenchyma cells and assist greatly in strengthening the stem. (Fig. 23.)

2. With the high power, the *laminated thick walls* of the cells.

3. The branching *canals* through these walls.

4. If the ends of canals of neighboring cells are in contact.

5. By careful focusing, the ends of these canals at the bottom of some of the cells.

Vines' Text Book of Bot., p. 133; Strasburger, p. 146; De-Bary, 132; Goodale, p. 63; Sedgwick and Wilson, p. 76.

IV and V Prosenchyma (in its widest sense.)

IV. PROSENCHYMA (proper) or FIBROUS Tissue.

(a) *Bast-cells*,—in the bark, (derived from *Phlœm.*)

(b) *Typical Wood-cells*—in the wood, (derived from *Xylem*).

V. Tracheary Tissue.

(c) *Tracheids*—like *Wood-cells* in *form*, and like vessels or *Tracheæ* in *structural markings.*

(d) *Tracheæ*—"Vessels" or "Ducts."

Type 1 includes *Dotted* and *Banded* Ducts, passing into *Pitted* and *Scalariform* Ducts by gradations.

Type 2 includes *Spiral*, *Annular*, and *Reticulated* Vessels and their gradation. See Goodale, p. 59; DeBary IV. *

Illustration: For Prosenchyma (in its widest sense.) Bark and Wood of Leatherwood (*Dirca palustris.*)

Preparation for (a). Remove the brown cuticle from a branch of *Leatherwood*, also the loose cells beneath. Make two or more very thin longitudinal sections of the bark, pulling one end of each section from the branch instead of cutting it. Many of the silky bast-fibers will thus be free at the end. Mount in water. Another mode, is to place bast tissue in nitric acid and potassium chlorate, and heat for a few minutes. The bast fibres can then be separated under a dissecting microscope. Use a 3-4 and then a 1-5 in. objective.

Observe: 1. The very long bast-fibres; trace out a free one, and measure its length.

2. What kind of cells, if any, occur with the bast.

3. The clear unmarked walls of bast; the tapering ends when not broken.

Preparation for (b) and (c). Make (1) very thin, tangential sections of wood; (2) very thin transections of the branch including wood and bark. Mount in water and stain with haematoxylin.

In the *longitudinal section:*

Observe: 1. The short unmarked, fusiform *wood fibres* or typical wood-cells whose ends over-lap. Measure the longest.

2. The fusiform cells marked by spiral lines, dots, bordered pits, etc.—*Tracheids;* see Goodale, Fig. 78, 79; Bessey, p. 81; Bastin, p. 162; Vines' Text Book of Bot., p. 182.

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* It will be observed that the classification of Prosenchyma tissue is not the one usually presented. It is believed that by adhering closely to this one in these studies, a correct idea can be obtained of the development of the more perfect forms of ducts from the simple cells. It is desirable that the classifications of others be compared and the points of difference noted.

3. Occasional *Ducts* (long tubes marked with bordered pits, bands, etc.)

4. Rectangular cells of medullary rays, if present. To what tissue-form do they belong?

In the *transection:*

OBSERVE: 1. Ends of wood-cells in very regular radial rows; thickness of their walls, etc.

2. The larger openings (ducts), marking each years growth.

3. Thin plates of medullary tissue.

4. The bark with ends of bast appearing.

5. Pith at center.

Bastin, p. 159; Goodale, pp. 88, 89.

Notice the brittleness of wood, and strength of bast, corresponding to the difference in the length of cells in each.

Usually wood-cells are less regularly distributed than in the above.

PREPARATION for (a), (b), (c), and (d). Make a radial longitudinal section (i. e., a longitudinal section passing the center of the pith). Around the pith is a sheath of *Spiral* or *Reticulated* vessels (see Type 1). In the woody tissue of the section are *Pitted* vessels (see Type 2).

OBSERVE: 1. The *Spiral Ducts.*

2. That the spiral appearance is due to the *thickened ridge* deposited on the inner surface of a thin-walled tube.

3. If there are any *Reticulated* or *Annular* vessels in the medullary sheath.

4. That the *Pitted* vessels are furnished with "bordered pits" —the outline of the pit or cavity being nearly circular, and the "lumen" or central aperture oblong.

Vines' Text Book of Bot., p. 134; Goodale, p. 85; Bessey, p. 74; Strasburger, p. 129.

V. Tracheary Tissue (continued).

Illustration: Sections of the stems of CURRANT, HORSE CHESTNUT, MOON SEED and GRAPE VINES, are to be mounted to serve as material for the study of both *Tracheids* and *Tracheae.* Note carefully the distinction between the two, the former being "*like wood cells in outline and like Vessels or Tracheae in structural markings.*" Read carefully DeBary, pp. 164–166.

Preparation: Suitable sections can be obtained by fastening small portions of these stems in the jaws of a microtome and sectioning with a stout razor. Several radial-longitudinal sections of each should be obtained in order to be certain of including the proper portion (xylem) of the fibro-vascular bundle.

Other material than that suggested above can be used, but such common plants have been selected as are found to show the desired structure. These specimens should be examined with reference to the study of the general forms indicated in the illustrations of *Tracheae* which follow. Vines' Text Book of Bot., p. 135; Goodale, p. 82; DeBary, p. 165.

The Tracheids of Coniferae.

Illustration: Wood of White Pine (*Pinus Strobus*). The seasoned sap-wood of a young Pine answers the best for the study.

Preparation First: Make several thin longitudinal sections at *right angles* to the "grain" (the annual layers.) Mount in water.

Observe: 1. The *Tracheids*, oblong and fusiform like wood fibres, but showing "*bordered pits*" at intervals. This form of Tracheid is found throughout the Gymnosperms, fossil and living.

2. The *outer* and *inner ring* of the bordered pit. By focusing, the inner ring or outline of the *lumen* on the opposite side of the pit may be seen.

3. The rows of rectangular cells occasionally crossing the Tracheids at right angles. These are portions of the *Medullary Rays*.

Preparation Second: (1.) Make several thin longitudinal sections parallel to the "grain." (2.) Make several thin cross-sections. Mount (1) and (2) in water under the same cover.

Observe in (1.) 1. The small cavities, occurring along the common-wall of two Tracheids. These are single and lens-shaped —the bordered pits seen in section.

2. Look for the *middle lamella* or "limiting membrane" which originally separated the two halves of the pit and was continuous with the common-wall of the two Tracheids.

3. The row of roundish or angular cells—three to six in number occasionally seen between Tracheids. These are larger

than the sectioned pits and are the cells of the *Medullary Rays* in cross-section.

Observe in (2): 1. The form of the *Tracheids* in cross-section.

2. The "*middle-lamella*" in the cell-wall of each.

3. The *Bordered-pits* and *Medullary Rays* in the section.

4. The *Resin-passages* in section. These are large openings surrounded by irregular thin-walled cells containing resin. These passages often occur running transversely, following the larger medullary rays. (Fig. 14.)

5. The *annual layers* of tissue (seen best with a low power) marked by alternating layers of larger and smaller cells, and crossed at right angles by the Medullary rays. (Strasburger pp. 115, 128a.)

6. The entire absence of true *Tracheae*, these being found in Gymnosperms only next the pith. In structure *Tracheids* seem to be intermediate between *Fibrous tissue* and *Tracheae*. Bessey, pp. 25, 26; Goodale, p. 83; DeBary, pp. 159, 160; Vines' Text Book of Bot., p. 200; Strasburger, p. 56.

V. (d) Tracheae.

Illustration: Sections of stems for the study of Tracheae,—Vessels or Ducts. The following general forms will be examined:

(a) *Dotted.* (c) *Spiral.* (e) *Scalariform.*
(b) *Pitted.* (d) *Reticulated.* (f) *Annular.*

For these studies the woody tissue is to be treated as directed for the previous illustration, but in the case of herbaceous stems the tissue should be hardened in alcohol and carried through the method for mounting, outlined on p. 21.

Preparation First: (a) Longisection of Grape vine stem.

Observe: 1. In the region of the section containing the fibro-vascular tissue, the large well developed *ducts* with *pitted surfaces*.

2. These dots are true openings allowing free communication between contiguous cells. Goodale, p. 29; Bastin, p. 162.

Preparation Second: (b) Radial longitudinal section of the stem of Castor Oil Bean, 1 c.m. in diameter (thin section mounted in water).

Observe: 1. Rectangular cells of *pith* and *cortex*.

2. In the vascular region; *large thick walled ducts*, with the surface covered with *bordered pits*.

3. The *orifice* of the pit, consisting of two "*acutely diverging lamellae.*" De Bary, p. 167; Sachs' Physiology, p. 137; Bessey, p. 27.

PREPARATION THIRD: (c), (d) and (e) Radial longitudinal section of the stem of BEGONIA, BEAN, BANANA, or better the material used in PREPARATION SECOND. In this preparation,

OBSERVE: 1. Large thin walled ducts with thickened *spiral markings.*

2. At various places the *spiral bands* broken loose from the wall of the duct.

3. In portions of the preparation some ducts may appear in longisection, in which case, the ends of the thickened bands may be clearly seen.

4. The *steepness* and *direction* of coils, also the number of bands.

5. Their *branching* at various places, often forming in some vessels a reticulation. This is the origin of the *reticulated ducts.* Excellent material for their illustration can also be found in the Cucurbitaceae and Impatiens.

6. Certain *ducts* with the broad openings, between the reticulations, arranged one above the other in the form of a ladder—*Scalariform Vessels.* Care must be taken to prevent confusing these with the pitted vessels which they closely resemble. De Bary, p. 158.

PREPARATION FOURTH: (f) Longisection of the stem of CORN. *Zea Mays.*

OBSERVE: 1. Lying next the large spiral vessels of the bundles, certain ducts strengthened by transverse thickenings in the walls in the form of rings,—*the Annular Vessels.* It is to be noted that these are but a modification of the spiral ducts. De Bary, p. 156; Strasburger, p. 90; Bessey, p. 82; Sachs' Text Book of Bot., p. 114;—Physiology, pp. 91, 136.

A modified form of *Annular Tracheae* exists in the *Trabecular Duct*, which has its walls strengthened by transverse bars across the cavity. Good illustrations of this can be found in the fibro-vascular bundles of the *Juniper leaf.* Bastin, p. 163; De Bary, p. 156.

VI. Sieve Tissue.

Illustration: Stem of Cucurbitaceae (Cucumber or Pumpkin).

PREPARATION: This material should be hardened in alcohol and treated as directed on p. 21. Both longitudinal and transverse sections are to be cut, stained with haematoxylin, and several of each permanently mounted. In the longisection, portions must be secured that contain some of the fibro-vascular tissue. In the latter section on the outer and inner edge of the bundle,

OBSERVE: 1. The thin walled *sieve tubes*, recognized by their *sieve like plates*, forming septa at the ends, and not infrequently on the lateral walls.

2. The *protoplasmic contents* of the tubes, frequently denser near the ends.

3. The structure of the *sieve plates*.

4. The *sieve pores*, of varying sizes, usually largest at the center.

5. A clear glistening bluish mass surrounding the sieve plate,—*the callus plate*.

6. A mass of *slime* collected at the end of the tube, with a portion of it projecting through the pores of the sieve. To a mount of fresh tissue add *sulphuric acid*, and observe its effects on the plates.

In the transection:

OBSERVE: 1. The *fibro vacular bundles* arranged regularly around the peripheral portion of the stem.

2. Two nearly circular or crescent shaped masses of tissue, (stained with haematoxylin a dark purple) one on the axial and one on the peripheral side of the bundle,—*The Phloem*.

3. By careful focusing upon some of the larger of the openings in these areas, *sieve plates*, forming septa.

Goodale, p. 92; Vines' Text Book of Bot., p. 136; DeBary, p. 172; Strasburger, p. 121; Annales des Sciences Naturelles, (Bot.) Vol. X., pp. 193–324; Prings. Jahrb., 21, 253–292.

7. Laticiferous Tissue.

1. *Latex cells.*
2. *Latex vessels.*

Illustration: For simple LATEX CELLS.

Stems or Petioles of EUPHORBIACEAE or ASCLEPIADACEAE.

PREPARATION FIRST: Make longitudinal sections of the stem or petiole of a *Euphorbia*, and mount in water.

OBSERVE: 1. Simple or branched cells or *coenocytes*, *latex cells*—ramifying the various parts of the preparation, and filled with a dense milky juice—*latex*.

2. The very thin walls of the cells and granular nature of the contents. The *latex* consists of a watery fluid with various *albuminoids*, *organic acids*, or *alkaloids*, in solution. The suspended matter may consist of *proteid compounds*, accompanied sometimes with *starch grains*.

LATEX TUBES OR VESSELS.

Illustration: Stem or Petiole of *Chelidonium majus* (Celandine) *Stylophorum diphyllum*, (Poppy.)

In sections obtained as for the previous study:

OBSERVE: 1. Thin walled *profusely branched tubes*, often anastomosing, and extending through the various tissues of the stem. These *latex tubes* are formed from rows of cells which become united by the absorption of the partition between, or by its perforation to allow free communication. The walls of these cells are usually thin, but frequently become thickened in the form of striations.

2. The effect of iodine on the latex tissue.

Goodale, p. 94; Vines' Text Book of Bot., p. 141; DeBary, p. 189: Bessey, p. 75; Strasburger, p. 104: Sachs' Text Book, p. 86-7.

Glands and Water Pores.

GLANDS.

Illustration First: SUBEPIDERMAL GLANDS of the "LEMON SKIN."

PREPARATION FIRST: Harden, section, and mount a piece of "*lemon peel*," after the method on p. 21.

OBSERVE: 1. Small cells of the epidermis, often containing crystals.

2. Near the outer portion of the section large cavities in the tissue—*The Glands* or *reservoirs of lysigenous origin*, i. e., formed by the breaking down of cells.

3. Thin-walled cells, with large nuclei, lining the cavities. In some cases the cells are much disorganized.

4. Crystals in the adjoining tissue.

5. Fragments of fibro-vascular bundles scattered through the preparation. (Fig. 15.)

PREPARATION SECOND: Make a tangential section and compare with the previous study.

Illustration Second: GLANDS in the leaf of *Eucalyptus.*

PREPARATION FIRST: Transections of a mature leaf can be prepared as directed for the previous study.

OBSERVE: 1. Large *glands* located on both upper and lower sides of the section.

2. The *cavity* of the gland.

3. The large thin-walled cells, partly disorganized, lining the gland, and the smaller thicker-walled ones just outside.

4. The *mesophyll* and *palisade cells* surrounding the gland.

5. The flattened epidermal cells above.

PREPARATION SECOND: Make transections of the young leaf of *Eucalyptus* and trace the formation of the gland, which results from the breaking down and absorption of the *mother cells.* Strasburger, p. 164, DeBary, p. 201, Vine's Text Book of Botany, p. 40, Goodale, p. 98.

The Resin Ducts of *Pinus* were examined in the study of the *Tracheids of Coniferae.*

Water Pores.

Illustration: LEAF TOOTH from *Fuchsia.*

PREPARATION: Several sections should be made serially, and must include those through the apex of the leaf tooth. (p. 35.)

In the section passing through the center of the tooth:

OBSERVE: 1. The *club-like enlargement* at the outer edge.

2. The epidermis, consisting of small cells, interrupted at the apex and forming a circular opening—*the pore.*

3. Two or three layers of chlorophyll bearing cells beneath the epidermis.

4. The large region of the center of the section occupied by the *spiral marked tracheids.*

5. The *water cavity* between the pore and the long *parenchyma*

cells beneath. The section should be preserved for reference in connection with the study of the leaf structure, of which this is a type of a very large group.

Bessey, p. 105, DeBary, p. 52, Vine's Plant Phys., p. 91.

D. MERISTEM TISSUE.

MERISTEM, or GENERATING TISSUE, is usually classified under two heads, viz:—

I. **Primary Meristem,** giving rise to the PRIMARY STRUCTURE in plants, such as young cellular tissue, and found at the apices of young thallomes, stems and roots, and at the apex and base of young leaves.

II. **Secondary Meristem,** or CAMBIUM, giving rise to the SECONDARY STRUCTURE, as the thickening growth of stems, roots of DICOTYLEDONS, or EXOGENS.

The SECONDARY MERISTEM, as PROCAMBIUM, forms a part of every fibro-vascular bundle in its earliest stage.

The Primary Meristem.

TYPE A. Of a SINGLE APICAL OR INITIAL CELL. Found in most of the higher CRYPTOGAMIA.

TYPE B. Of a GROUP OF INITIAL CELLS. This is characteristic of PHANEROGAMS.

Illustration, Type B: Root from a cultivated *Hyacinth* grown in a jar of water.

PREPARATION FIRST: Trans- and longitudinal sections prepared after the method on p. 21, also for serial sections, p. 35. (Fig. 16.)

In the longitudinal section, observe the general arrangement of the different layers of cells, viz:

1. That the several series of cells converge into a rounded cone at a point just back of the apex. This point is the center of the so-called "*Initial Group*" which constitutes the Primary Meristem. It is impossible to say just how far meristematic cells extend from this point.

2. The *Calyptrogen*, a row of cells on the apical side of the Initial Group, producing rows of cuboidal cells radiating towards the apex of the root, the outer ones becoming rounded and loosely coherent.

3. The *Dermatogen*, arising from the Initial Group curving to the right and left and ultimately producing a double layer of cuboidal or flattish cells bounding the section on each side. In large roots it consists of more than two rows of cells.

4. The *Plerome*, axial rows of oblong or cuboidal cells forming a central band immediately back of the Initial Group.

5. The *Periblem*—thin-walled cells of various forms in several rows between the Plerome and Dermatogen.

6. That the differentation into the above named layers occurs at a very early period in the development of the root-tissues. They retain their original distinctness after passing over into permanent tissue when they are named as follows:

The Calyptrogen gives rise to the *Root-Cap*.

The Dermatogen gives rise to the *Epidermis*.

The Periblem gives rise to the *Cortex* or *Cortical Parenchyma*.

The Plerome gives rise to the *Axial* or *Central Cylinder*.

7. The first appearance of *Spiral Vessels* on the right and left margin of the Plerome. Trace them backwards. They represent the beginnings of the Fibro-Vascular Bundles which always arise within the Plerome in both stem and root.

8. The two rows of cells on the peripheral side of the Spiral Vessels. The inner (the *Pericambium*) belongs to the Plerome, the outer (the *Endodermis*) belongs to the Periblem or Cortex. The Endodermis is sometimes called the "Plerome-sheath" or "Bundle-sheath."

9. The peripheral layers of cells of the Plerome itself, extending back from the Initial Group to the beginning of the Fibro-Vascular Bundles. These are termed collectively the *Procambium*, —as it is from such cells that the tissues of the Bundles themselves arise by fission and differentiation.

10. Occasional dark lines along the lateral walls of the Cortex cells. These are due to cylinders of air in narrow intercellular spaces. (See 2 under observations below on the cross-section.)

11. The *Raphides* or Needle-shaped Crystals, in black bundles in many cells of the *Cortex*, and in this tissue only.

To a fresh section apply the tests for Crystals given on p. 3.

In the Cross-Section: (Fig. 17.)

OBSERVE: 1. The *Epidermis*, and outer zone of two or three layers of cells; form of latter.

2. The zone of the *Cortex* cells, with frequent small intercellular spaces. (See obs. 10 above).

3. The Endodermis layer of the Cortex with a dark spot on the common wall between adjoining cells, due to a minute fold in the wall.

4. The *Axial Cylinder*.

a. The *Pericambial layer*.

b. The Bundles five to ten in number; the *Xylem* mass (that including the large duct openings) alternating with the thin-walled *Phloem*, after the usual plan in roots. See Fig. of *Acorus* in Sachs' Botany, p. 115; De Bary, pp. 10,348; Strasburger, p. 184; Goodale, pp. 105, 112; Vines' Text Book of Bot., p. 147.

Make a sketch of a cross-section and the longitudinal section, showing the zones and layers above mentioned. Also an enlarged drawing of two xylem rays, the intermediate Phloem, Pericambium, and Endodermal layers.

A comparison should be made with the roots of a Dicotyledon (Radish, Pea, etc.), treated after the same manner as directed above.

With reference to the development of the various parts of the root from *distinct initial groups*, several types have been described by Janczewski, Flahault, and others. See Goodale, p. 107; De-Bary, p. 7.

Type A.

A SINGLE APICAL OR INITIAL CELL.

Illustration: Root of FERN (Pteris) or EQUISETUM.

PREPARATION: The same as for the previous study, except that serial logitudinal sections *must* be made in order to insure the presence of the desired parts. See p. 35. In the median section, OBSERVE: 1. The cone-shaped *root cap* covering the tip of the root, not unlike the one in type B.

2. Just beneath the cap and at the center of the root, the

large *trilateral pyramidal cell* with its convex base turned toward the root-cap,—*the apical cell.*

3. A transverse partition cutting off the outermost portion, which becomes the *initial cell* of the root-cap,—*Dermatogen.*

4. Segments cut from the innermost faces of the cell and, by longitudinal partition, developing into the tissues of the *Plerome and Periblem.*

Strasburger, p. 188; Vines' Text Book of Bot., p. 150; DeBary, p. 18.

In the transection, OBSERVE: 1. The irregularly thickened walls of the *epidermis.*

2. The *cortex* consisting, on the peripherial portion, of dark brown parenchyma and merging into sclerenchyma toward the plerome.

3. The *bundle sheath* between the cortex and fibro-vacular bundle.

4. The *pericambium*, a narrow sheath (just inside the bundle sheath) of parenchyma cells filled with protoplasm.

5. The regular *radial bundle* of the root.

If time will admit a comparative study should be made of the *apex* of the *stems* of Monocots, Dicots, and higher Cryptogams.

The preparation of the tissues for study would be the same as that recommended for the roots. DeBary, p. 19; Strasburger, p. 177; Vines' Text Book of Bot., p. 146.

Fibro-Vacular Bundles.

The following general classes are noted:

A. *Collateral.*

B. *Bicollateral.*

C. *Radial.*

D. *Concentric.*

Illustration A: Stem of BEGONIA, GERANIUM, MOON SEED Vine, or SMILAX.

PREPARATION FIRST: The herbaceous stems should be prepared in the usual way, the woody ones may be sectioned by placing them between pieces of cork in the jaws of a microtome. Transection and longisection should be mounted together.

In the preparation of the Moon Seed Vine, (*Menispermum Canadense*) a stem of *one year's* growth should be selected. (Fig. 27.)

Examine with a hand lens, and observe the general management of the *tissue systems* in a cross-section.

1. *Pith*, (Fundamental Tissue.)
2. *Fibro-vascular bundles*, (outline of each is nearly circular).
3. *Epidermis.*

OBSERVE: 1. The *Epidermis;* The external walls of its cells being thickened very greatly, and a light olive in color, the inner and lateral walls being of ordinary thickness and the cell cavity small.

2. The *Cortical Parenchyma;* of two kinds of cells, both containing chlorophyll.

(a) The *outer* are regular, closely packed and thick-walled.

(b) The *inner* are thinner-walled, larger and looser, passing into the oblong cells of the Medullary Rays.

3. The *Fibro-Vascular* Bundles:

The *Phlœm* is sharply distinguished from the *Xylem*, forming a strictly *Collateral Bundle.*

In the *Phlœm:*

(a) The *Crescent-Shaped* band of thick-walled cells on the peripheral side of the Phlœm; the lamellate structure of the walls.

(b) The *squarish outline* of *Phlœm* cells adjoining the thick-walled *Xylem.* Some of them somewhat thickened (lignified).

(c) The larger cells lying between (a) and (b). They are irregular in outline, thin-walled and stain but slightly.

In the *Xylem:*

(d) The larger *duct openings* between which the smaller cells (tracheids, etc.,) appear.

(e) The thick-walled cells of the *axial* region of the xylem forming a continuous band, or "bundle sheath," bounding the axial side of the fibro-vascular bundles.

(f) Between the *Xylem* and *Phloem* several of narrow, thin-walled cells—*the Cambium.* This is the region of growth, in secondary thickenings.

In the *radial-longitudinal sections* trace out the groups of

cells corresponding to those observed under the previous preparation, and demonstrate the *Tissue Forms* in each.

Drawings should be made sufficiently in detail to show the character of the above-mentioned groups of cells in both trans- and longitudinal section.

DeBary, pp. 319-339, Strasburger, p. 107, Bessey, p. 117, Vine's Text Book of Bot., pp. 174-182.

Compare with closed collateral bundle of a monocot, (Fig. 19, 28.)

Illustration B: From the stem of *Cucurbit Pepo.*

Preparation First: Radial longitudinal and transverse sections are to be prepared.

Observe: 1. The *Fibro-Vascular Bundles*: the smaller ones opposite the external ridges of the stem; the larger alternating with the smaller, and occupying the ridges projecting into the central cavity of the stem. To the naked eye the bundles appear as ganglia of smaller cells.

2. The *larger openings* in the middle area of the larger bundles, 3 to 8 in number.

3. A group of *smaller openings* on the axial side of the larger ones. This middle area is the *xylem.*

4. Two nearly *semi-circular* or sometimes *crescent-shaped areas* of tissue, one on the *axial*, and one on the *peripheral*, side of the xylem. Both of these are masses of *Phloem.* Focusing upon the larger openings of these areas, *sieve-like* plates may be observed in some, forming septa. (Sachs' Text Book, p. 113.)

5. Form of cells in other parts of the bundle.

6. The thin-walled parenchyma, forming the greater part of the stem and surrounding all the bundles. This is the tissue of the *Fundamental system.*

7. The *Epidermis* of the Stem.

8. The "*Intra-Cortical Ring*":—a band of several layers of thick-walled cells, but faintly colored, and separated from the epidermis by slightly stained cells.

9. In the above-named slightly stained band note the separated areas of Collenchyma cells. Bessey, p. 30.

Collenchyma cells, as previously noted, are recognized by the *thickening* of the walls at the *angles.*

In the middle longitudinal section of one of the bundles:

OBSERVE: 1. The *Fundamental Tissue* (Parenchyma), on the axial side of the bundle beyond its limits.

In the Axial Mass of Phloem:

2. The *Sieve-Tubes;* known by the Sieve-like plates forming the septa or occasionally seen on the lateral walls. See Sachs', p. 113; Bessey, p. 78.

3. The *Protoplasm* enclosed in these *tubes.*

4. The *Fibrous-cells*, fusiform in outline, scattered among the Sieve-tubes or occurring in a band next the Spiral Ducts.

In the Xylem of the Bundle:

OBSERVE: 5. The *Spiral Ducts*—one narrow with a loose spiral; another broader with a close spiral, occasionally becoming reticulated.

6. A *Scalariform duct;* see Bessey, p. 27, (Fig. 18;) sometimes this approaches (in this plant) a Pitted Vessel in structure.

7. The *Pitted Vessels*—very broad.

a. The shallow pits on the walls in transverse rows showing a "border" and "lumen."

b. The *ridges* on the walls between rows of pits.

c. The frequent occurrence of *rings* marking the partially absorbed septa.

8. The thin-walled cambiform cells, with septa oblique or at right angles to the lateral walls. This layer separates the *Xylem* of the Bundle from the *Phloem* on the peripheral side of the bundle.

In these bundles it will be noted there exists a *permanent* band of *cambium*, which insures the continued growth of the vascular tissue. The bundle is therefore said to be an *open* one.

In the *closed* bundles the procambium tissue becomes differentiated into permanent wood and bast, and the bundle undergoes no further differentiation. The latter condition is the case in the stems and roots of most Monocots, and Pteridophytes. Vines' Text Book of Bot., p. 177. Strasburger, p. 83; Bessey, p. 121.

In the *Peripheral* Mass of Phloem:

9. Another group of *Sieve-Tubes.*

10. A few *Bast Fibres.*

Trace in the cross-section, the tissues, cells, etc., correspond-

ing to those observed in longitudinal section, particularly noticing what belongs to the *Phloem*, and what to the *Xylem*.

This *Bundle* is not only a *Collateral Bundle* (Bessey, p. 120), but a *Bicollateral Bundle*, (Fig. 20) i. e., one having a layer of *Phloem tissue* (Sieve-tubes, etc.), on the axial, as well as on the peripheral part of the Bundle. De Bary, p. 319.

Sketch so much of the *cross-section* as shall include one Bundle and the tissue extending from it to the Epidermis; also the middle *longitudinal section* studied. The drawings should be on a scale to show the characteristics of each tissue-form.

Illustration C: Radial Bundle in the root of Hyacinth, or Corn. (Figs. 16, 17, 21, 22.)

In the transection prepared in the usual way,

Observe: 1. The central portion of the root surrounded by the *Endodermis*, a single layer of cells easily recognized by the suberized thickening of the contiguous walls.

2. Just inside the *Endodermis*, arranged in a circle, 12-18 groups of thick-walled cells,—*vessels in section*. These, together with the *wood parenchyma*, constitute the *xylem* of the bundle.

3. Between these xylem areas, groups of thin-walled cells, with a few large sieve-tubes in section,—The *Phloem* of the Fibro-Vascular Bundle.

4. The large isodiametric cells of the *Pith*.

Vines' Text Book of Bot., pp. 165, 168; Bessey, p. 115; De-Bary, pp. 348-366; Goodale, p. 109.

Illustration D: Concentric Bundles from the Rhizome of *Pteris*.

Preparation First: Good sections can be obtained by fastening some of the material, hardened in alcohol, between two pieces of cork in the jaws of a microtome, and sectioning with a strong razor. The material must be kept wet during the operation, and after staining can be mounted directly in glycerin jelly, or after dehydrating and clearing, in balsam.

Observe: 1. Circular or lunar-shaped masses of whitish tissue scattered through the preparation,—*The Concentric Bundles.*

2. The well marked *bundle sheath*, composed of a row of ellipsoidal cells.

3. With the high power, the *xylem* of the bundle occupying

the center, and consisting of thick-walled *scalariform vessels* and *xylem parenchyma.*

4. Outside of the *xylem* and completely surrounding it, a band of tissue limited by the bundle sheath,—*The Phloem.* This part of the bundle consists of *cribose tissue*, and *phloem parenchyma.*

Make a longitudinal section of the bundle and identify in it, all of the tissues seen in the transection.

It is to be noted that in this bundle the xylem portion is completely enveloped by Phloem and both surrounded by the *bundle sheath.* The opposite arrangement of Phloem and Xylem is not infrequently found.

Illustration Second: Concentric Bundle in the Rhizome of Iris. (Fig. 23.)

Preparation: Transections may be cut free-hand or treated as previously directed for herbaceous stems. By carefully focusing on one of the smaller bundles,

Observe: 1. In the center, the thin-walled cells of the Phlœm with a few sieve plates here and there in the larger openings.

2. The large thick-walled cells *outside* of the Phlœm and surrounding it,—*the Xylem.*

Vines' Text Book of Bot., p. 175; Strasburger, p. 145; De-Bary; p. 339; Bessey, p. 107; Sedgwick and Wilson's Biology, pp. 72, 78.

E. TISSUE SYSTEM (of Sachs).

1. **Epidermal Tissue System.**
2. **Fibro-Vascular Tissue System.**
3. **Fundamental Tissue System.**

1. The Epidermal Tissue System.

Tissues Covering the EXPOSED SURFACES of Plants. Epidermal Cells and their Modifications.

Illustration First: EPIDERMAL CELLS, STOMATES, and TRICHOMES. For this study examine the notes and drawings made on p. 83.

Illustration Second: EPIDERMAL CELLS and FORMATION of STOMATES. A very young leaf of a "STONE CROP," (*Echeveria secunda-glauca*), or *Sedum ternatum*.

PREPARATION: With a razor make a thin section parallel to lower surface of leaf, extending from the middle to the base, and including the Epidermis and a little parenchyma. Mount sections in water.

OBSERVE: 1. The *epidermal cells* of irregular forms, without chlorophyll, the outline distinctly sinuous.

2. The *stomates*, oval or nearly circular when fully formed.

3. The *guard cells* of a stomate; lunate in form and containing grains of chlorophyll.

4. The *Pore* of the Stomate.

5. The several stages of development in the *young* or *forming stomates;* beginning with the *initial cell* and ending with the "mother cell" of the stomate just split into the two "guard cells." The various septa even the primary one may be distinguished from the original epidermal cell-walls by their straight or curved (not sinuous), lines.

6. The "*subsidiary cells*" surrounding the *stomate*. They are the result of various sub-divisions of the initial cell. Sachs' Bot., p. 103; Bessey, p. 101.

For the study of the more complex forms of stomates an examination should be made of those in the leaves of *Pinus* and *Cycas* and the stem of *Marchantia*. (Fig. 26.) Bot. Gazette, 1889, p. 76; DeBary, p. 72; Botanisches Centralblatt, xxiv, pp. 54, 85, 118, etc.

Illustration Third: (For Trichomes.) Leaf of *Shepherdia Canadensis*. (Fig. 24.)

PREPARATION: With a knife blade remove a mass of *scale-like trichomes*, 2 or 3 m. m. square, from the lower surface of the leaf and mount in alcohol, remove this by water, and mount permanently in glycerin jelly. Seal after a few hours.

Observe the *stellate* and *peltate* scale-like *trichomes*, all gradations of one type. In some cases the stalks of the trichomes may be seen.

Notice the roots of Indian Corn growing in water. The velvety covering of the roots consists of root hairs, which are *true trichomes*. (Fig. 12.) Goodale, p. 109; Vines' Text Book of Bot., p. 158; Sachs' Physiology, p. 260.

Illustration Fourth: STINGING HAIRS from NETTLE, *Urtica dioica*. (Fig. 25.)

PREPARATION: Make thin longitudinal sections of the stem that shall include one of the stinging hairs.

OBSERVE: 1. The firm-walled *unicellular hair* with a *sharp apex*, and broad swelling at the base.

2. The extension of the hair into a *cup-like receptacle* formed by the tissue of the stem. In the mature hair, the *conical base* has been lifted on a column of tissue, formed from the hypodermal layer yet covered by the epidermis.

3. The strongly *silicious wall* of the hair often showing *black striations*.

4. The cell contents. The cells also contain a strong *acid poison* which enters the wound made by bringing an object into forcible contact with the trichome.

Further studies of modified hairs may be made with profit if the material is at hand. Excellent illustrations are found in the

DIGESTIVE HAIRS of many insectivorous plants, GLANDULAR HAIRS on the scale of the winter bud of *Aesculus Hippocastanum*, and various kinds of BARBED HAIRS from *Mentzelia ornata*.

Strasburger, pp. 72–82; DeBary, pp. 59, 89, 94, 95; Bastin, pp. 42–47.

It will be noticed that true plant hairs (trichomes) are but a part of the epidermis which has become differentiated, while thorns are modified branches, and are connected with the vascular system of the plant, e. g., thorns of locust.

For the study of Water Pores, in part modifications of the epidermis, the leaves of *Tropaeolum*, *Aconitum* or *Ficus elastica*, furnish excellent material, but are not always readily accessible. It will be sufficient in this connection to refer to the study made of the leaf tooth of *Fuchsia*, p. 97.

Additional studies could profitably be made of many of the modifications which the epidermis undergoes, but it is believed that enough has already been given, to familiarize the student with the more important characters of this interesting tissue system.

2. Fibro Vascular System.

3. Fundamental System.

Illustration: These Systems should be considered in the following groups of plants: (1) EXOGENOUS STEMS, herbaceous (*Begonia*), and woody (Moon Seed Vine). (2). ENDOGENOUS STEMS, herbaceous (Corn), and woody (*Smilax hispida*). (3). CONIFERAE. (4). VASCULAR CRYPTOGAMS. (Figs. 16, 17, 27, and 28).

Since studies have previously been made of the *elements* of the Fibro-vascular bundle, in each of the groups as outlined, it is only necessary in the brief course here included to examine the *distribution* of the tissues in each of the cases mentioned, together with the relations of the three tissue systems. To this end, careful series should be made of the *longisection* and *transection* of stems from the plants indicated. De Bary, p. 232; Goodale, pp. 126–135; Vines' Text Book of Bot., p. 170.

It must be remembered that the VASCULAR SYSTEM of ROOTS, LEAVES, AND THEIR MODIFICATIONS should be included in the consideration of this subject.

By examination of the material indicated make outline sketches

that shall represent the arrangement of the three systems in the types indicated.

The *Fundamental Tissue System* includes all tissues not forming a part of the epidermis and its modifications, nor included in the Fibro-vascular bundles. Examples of this system are found in the CORTEX, MEDULLARY RAYS, and PITH of stems; CORTEX, PITH, and ENDODERMIS OF ROOTS, and the MESOPHYLL OF LEAVES. Bessey, pp. 106, 122. (Figs. 14, 16, 27, and 28).

F. SECONDARY THICKENING.

This is usually produced by the differentiation and development of tissue, by means of Open Fibro-Vascular Bundles.

I. In Dycotyledonous Stems.

Illustration: Stem of Moon Seed Vine. (Fig. 27.)

Preparation First: Refer to the permanent preparation made from the stem of this plant representing one year's growth. P. 103.

Preparation Second: Stem of the Moon Seed Vine of two or more years' growth. Prepare transections and longitudinal sections corresponding to those made for Preparation First.

Observe: 1. The changes in aspect when compared with Preparation First; (a) The several *annular layers* of wood formed from the xylem; (b) each layer in any bundle having one of corresponding thickness in the bundles on the right and left.

The Annular Layers; (c) The distribution of spiral and woody vessels; (d) Of woody fibre; (e) The slight changes in the Phloem region of the bundles.

2. The cambium ring, of thin-walled cells forming by their division new tissue. (1) Xylem. (2) Phloem. (3) Medullary. (4) New Cambium.

This ring of cambium is divided into two portions, (1) *Fascicular*, that included in the fibro-vascular bundle. (2) *Interfascicular*, that between the bundles and included in the *Medullary Rays*.

4. The large *Medullary Rays*, composed of cells whose walls have not become thickened, (contrary to the general rule in Medullary Rays of several years' growth.)

5. The *isolation* of the bundles, which still retain their individuality, on account of the large Medullary Rays.

6. That the stem has nevertheless been thickened, after the usual mode in Dicotyledons, i. e., by the addition of a new layer, in a uniform manner, to the bundles.

Compare the sections of an Oak or Beech twig where the individuality of the bundles is not so well preserved. Sachs' Text Book, p. 129; Vines' Text Book of Bot., pp. 167, 191; Goodale, p. 137. Make sketches of the transection in Preparation Second to illustrate the changes which have taken place in the thickening process.

II. In Monocotyledonous Stems.

Illustration: Stems of *Smilax hispida.* Sections from (1) one year old, (2) several years old. (Fig. 28.)

It is to be noted that there is no *cambium ring* in Endogens, and the bundles being closed, i. e., without permanent cambium, are not able to increase, by secondary growth, the size of the stem. Compare the bundles in the stems of the two preparations and note the changes, if any, that have occurred during the development of the plant. In many Monocotyledons there exists a ring of meristematic tissue in the cortex, just beneath the epidermis, where new closed bundles are formed, thus enabling the stems to increase in size.

Vines' Text Book, p. 202; Goodale, p. 135; DeBary, p. 618; Annals of Botany, Vol. VII, p. 21.

Make transections of the stems of *Pinus* which shall include those from one to several years' growth. Note the changes that have taken place in the thickening of the stem.

Strasburger, pp. 115, 116; Vines' Text Book, p. 193; DeBary, pp. 461, 567.

The study of the development of Fern Stems will not be included in this course. DeBary, p. 623.

Secondary Thickening in Roots.

I. Monocotyledons: As in the case of Monocotyledonous Stems, so with most of their roots, no true *cambium zone* is found, but the roots retain their primary differentiation throughout their existence, except that the tissue may become firm by age.

Illustration: The Roots of Orchidaceae, Cyperaceae, or Liliaceae.

Preparation: Make transections of the young and old roots of any of these plants, and note the changes that may have occurred. DeBary, pp. 360–362, 618; Annals of Botany, Vol. 7, p. 21.

II. Dicotyledons: The changes in the primary structure of Dicotyledonous roots usually take place soon after the arrangement of the primary tissues, and continue during the activities of the plant.

Illustration: Roots of various sizes from the Ranunculaceae and Sapindaceae.

Preparation: Treat the material as directed for herbaceous and woody stems.

Compare the sections of different ages, and observe that the *secondary thickening* has brought about the following changes:—

1. Formation of a *cambium ring*, by the longitudinal division of cells on the axial side of the Phloem areas, and the extension of the same laterally to their union with the next areas, over the ends of the xylem rays. VanTieghem, Ann. Sci. Nat., 5 ser., Tom. XIII, p. 185, pl. 3, 4, 8.

2. *Irregular outline* of the *cambium ring* corresponding to to the *sinuses* between the *xylem areas.*

3. Disappearance of these *sinuses* in the older roots, and the nearly circular outline of the *cambium ring.*

4. After these changes, the thickening corresponds to that in the stem.

5. In the *mature roots;* the wood (xylem), *bast* (phloem), and alternating with these plates of parenchyma,—*the medullary rays.* DeBary, p. 473.

VASCULAR SYSTEM OF LEAVES.

The arrangement of the Vascular System in the leaf, with its various modifications, or variations in the different groups of plants, presents a subject too extended to be treated in this brief course.

Read carefully, Strasburger, pp. 160–169; DeBary, pp. 296–307, 372–373; Goodale, p. 155.

In structure the Fibro-Vascular Bundles are much the same

as those in the stem, but are often surrounded by a layer of thick walled fibrous tissue, which adds much to their strength.

Treat a small thin leaf (*Oxalis*), with KOH and mount. The preparation may be made permanent by washing *thoroughly* with water and mounting in glycerin jelly.

OBSERVE: 1. The *anastomosing Fibro-Vascular bundles*, indicating their full development.

2. The *free ends* of the bundles, consisting of the *spiral tracheids*, in close contact with the mesophyll cells of the leaf.

3. The appearance of one of the larger bundles in transection.

LENTICELS.

Illustration: Elliptical wart-like thickenings on the stem of ELDER (*Sambucus Canadensis*), or MOON SEED VINE (*Menispermum Canadense*).

PREPARATION FIRST: Fasten pieces of the stem in the jaws of a hand microtome, and make several thin transections through a lenticel. Mount in water and OBSERVE: 1. The *ruptured epidermis* of the stem.

2. Beneath this the true *cork cells*, not closely united but provided with *inter-cellular spaces*, which allow free communication between the inner tissue and the air outside.

3. Below the loose cork cells, a layer of thin-walled rectangular ones, constituting the true meristematic region of the cork,—*the Phellogen*. The continued development of this tissue produces in time a ring of cork which breaks away from the stem in the form of the rough corky bark.

4. Inside of the *Phellogen* and formed from it is the *Phelloderm*, a layer of cells regular in outline, containing protoplasm, and in many cases chlorophyll. These cells resemble those of the *primary cortex*, and are formed to replace any of the *cortical tissue*, that may have been obliterated by the pressure resulting from the rapid growth of firmer tissue beneath.

Vines' Text Book of Bot., p. 212; Goodale, p. 151; DeBary, p. 560; Strasburger, p. 153.

Plate I.

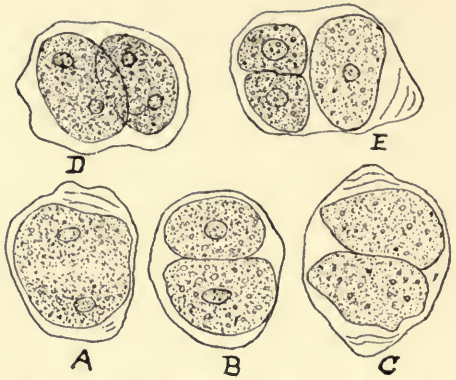

FIG. 9. Mother cells with developing pollen grains from *Funkia ovata* The illustration shows successive figures from A - E and represents, from the first division of the contents of the mother cell, (A), to near the formation of the four pollen grains, (E). (x550). After Sachs.

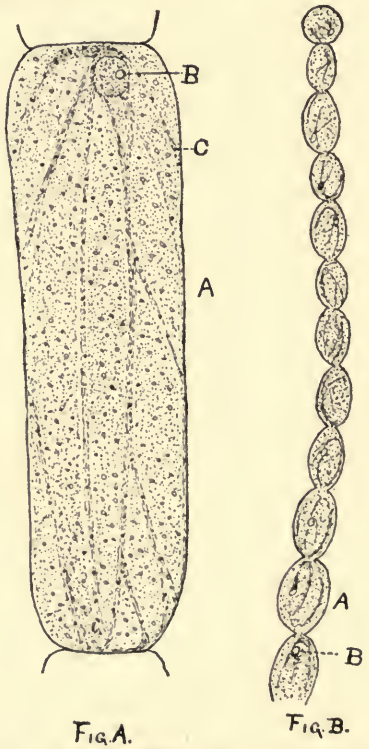

FIG. 10. Stamen hair of *Tradescantia*. Fig. B. Stamen hair showing general form and relations of cells. A, cell of proximal end; B, nucleus. (x20.) Fig. A. Cell much enlarged. A, outer wall of cell; B, nucleus and contained nucleolus; C, stream of living protoplasm. (x280.)

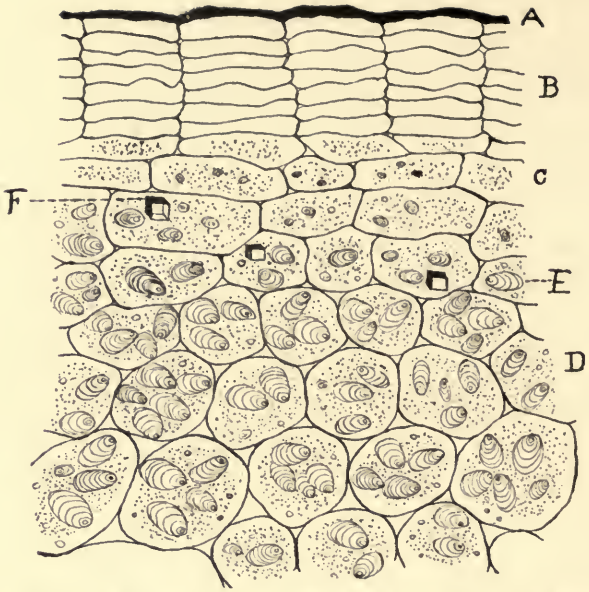

FIG. 11. Section of Potato tuber. *Solanum tuberosum*. A, brown epidermis; B, rectangular cells just beneath epidermis; C, hypodermal cells containing *crystalloids* (F); D, starch bearing parenchyma; E, starch grains. (x80). (Modified after Landois and Stirling.)

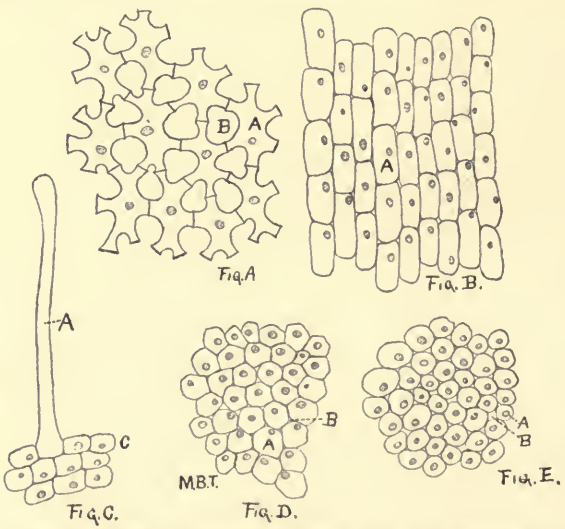

FIG. 12. Various forms of parenchyma cells. Fig. A. Stellate from the stem of *Pontederia;* Fig. B. Ellipsoidal and rectangular from the root of *Hyacinth;* Fig. C. Cylindrical from the *Hyacinth* root, (a trichome, or root hair); Fig. D. and E. Isodiametric and globose from the cortex and pith of a *Geranium* stems. A. Cell cavity; B. Intercellular space. (About x100.)

Plate III.

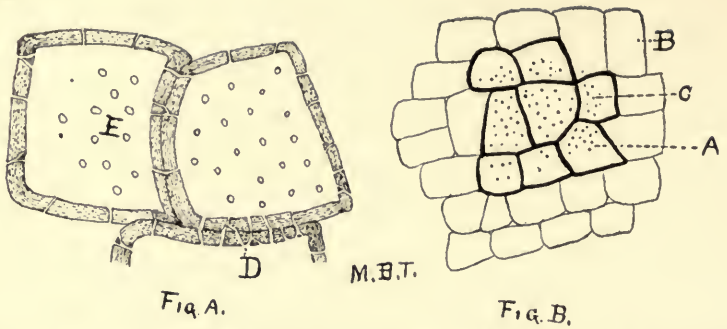

FIG. 13. "Grit cells," (sclerenchyma), from the *Dahlia* root. A, Group of cells; B, thin walled cells surrounding the sclerenchyma; C and E, opening of canals in the cell wall; D, canals in section. Fig. A. (x300.) Fig. B. (x100.)

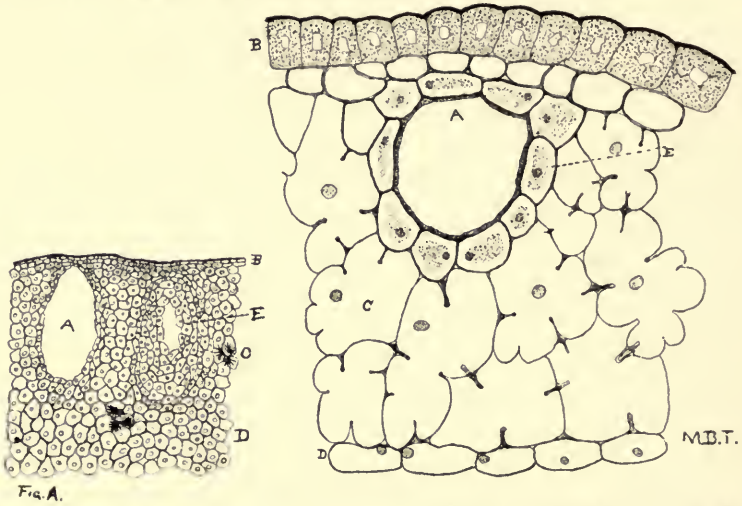

FIG. 14. Transection of the leaf of *Pinus sylvestris* A, Cavity of the gland; B, thick walled epidermis; C, parenchyma of the mesophyll; D, bundle sheath; E, large thin-walled cells lining the gland. (x300.)

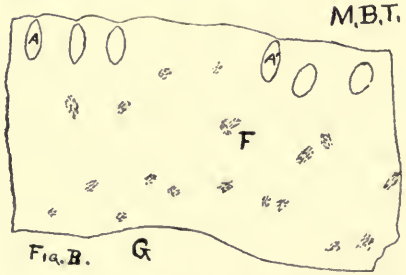

FIG. 15. Transection of "Lemon peel." Fig. B, Slightly enlarged to show general relations of parts. (x5); F, Fragments of fibro-vascular bundles; G, Interior of the "peel." (x30.) Fig. A, More highly magnified to show the character of a gland and the surrounding parts; A, Cavity of the gland; B, epidermis; C, crystals, abundant in the parenchyma (D), surrounding the gland; E, thin-walled cells, usually more or less disorganized, lining the gland.

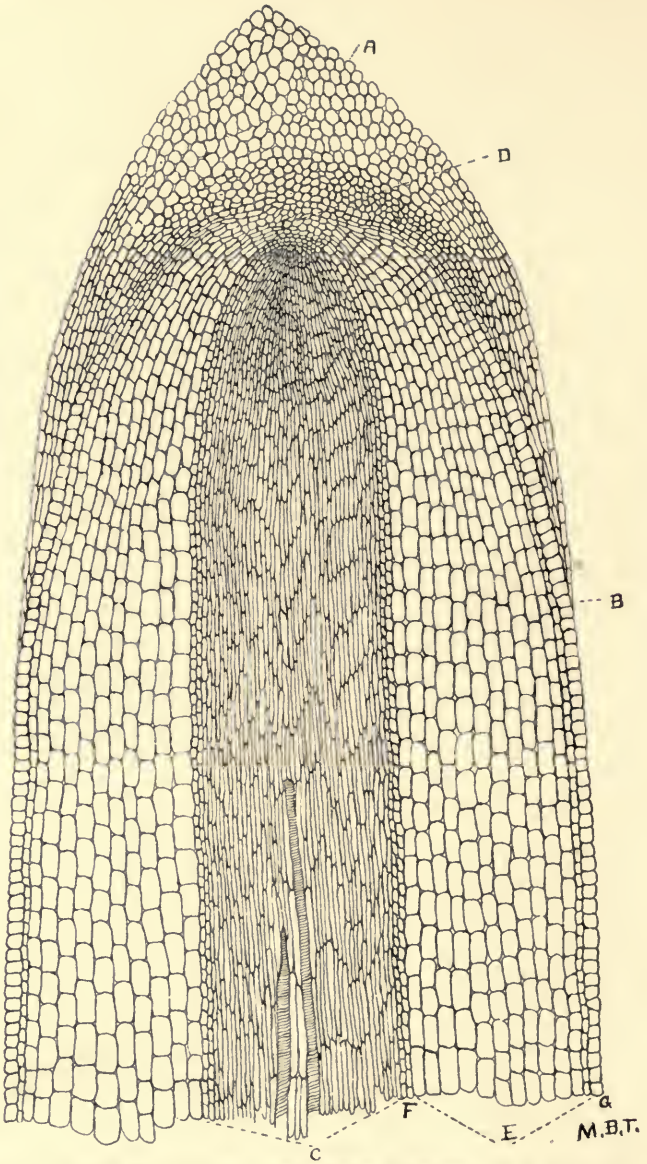

FIG. 16. Longisection of root, *Cypripedium pubescens*. A, B, Root-cap, completely covering the tip of the root; C, central or plerome cylinder, showing the thin-walled procambium cells which later develop into the elements of the fibro-vascular bundle; D, initial group of meristematic cells from which originates the various tissues of the root; E, cortex developing from the periblem of the meristematic region at the tip of the root; F, plerome or bundle sheath composed of a single layer of modified parenchyma cells, having the walls uniformly suberized but folded or thickened at their points of contact; G, epidermis having its origin in the dermatogen at the apex just outside of the periblem. (x30.)

Plate V.

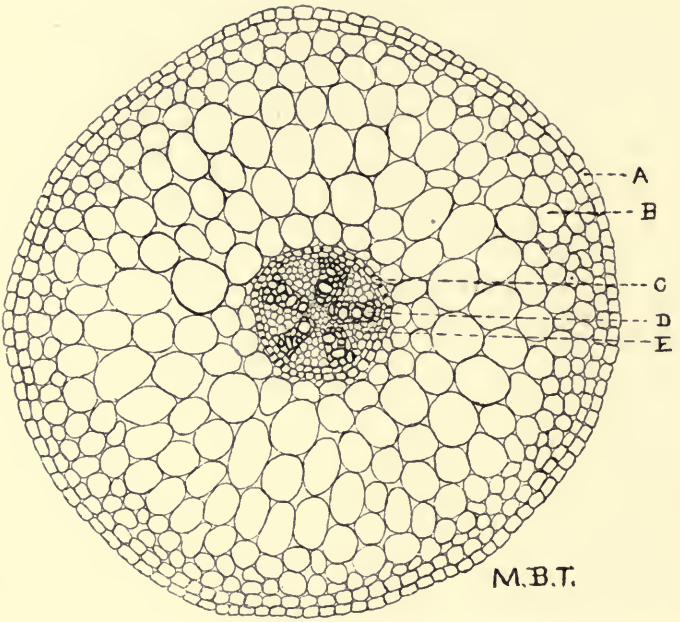

FIG. 17. Transection of the root of *Cypripedium pubescens*. A. epidermis; B, cortex; C, bundle sheath; D, xyiem ray; E, phloem. See Fig. 16 for parts in longisection. (x30).

Plate VI.

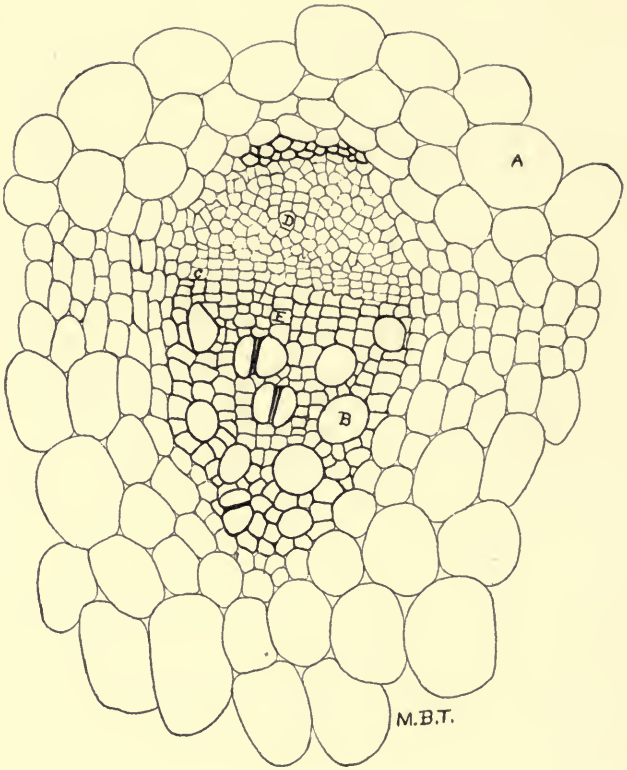

FIG. 18. Transection of an open, collateral fibro-vascular bundle from the stem of *Begonia nitida*. A, thin-walled cortical parenchyma surrounding the bundle; B, large thick-walled vessels of the xylem; C, thin-walled cells of the cambium; D, sieve duct of the phloem; E, wood parenchyma of the xylem. (x350.)

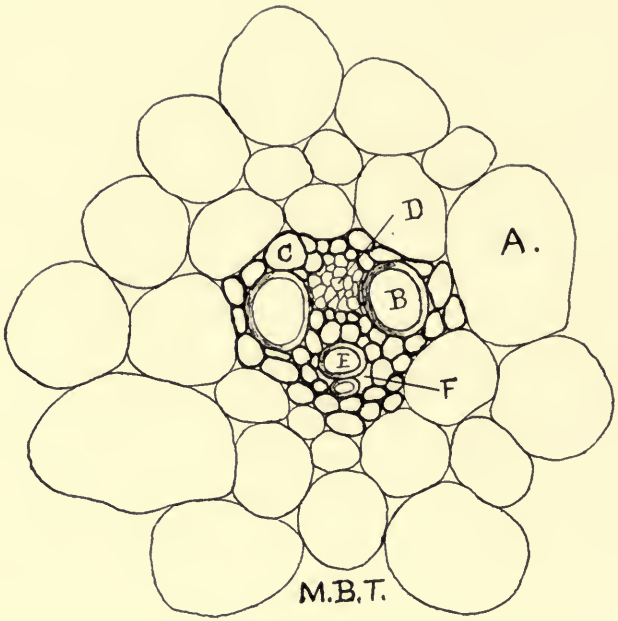

FIG. 19. Tranverse section of a closed, collateral vascular bundle from the stem of Indian Corn, *Zea Mays*. A, thin-walled parenchyma surrounding the bundle; B, large pitted vessels in section; C, sclerenchymatous cells forming a sheath about the bundle; D, phloem of the bundle, with sieve-tubes and bast-fibres; E, spiral or annular vessel in section; F, intercellular space of lysigenous origin. (x200).

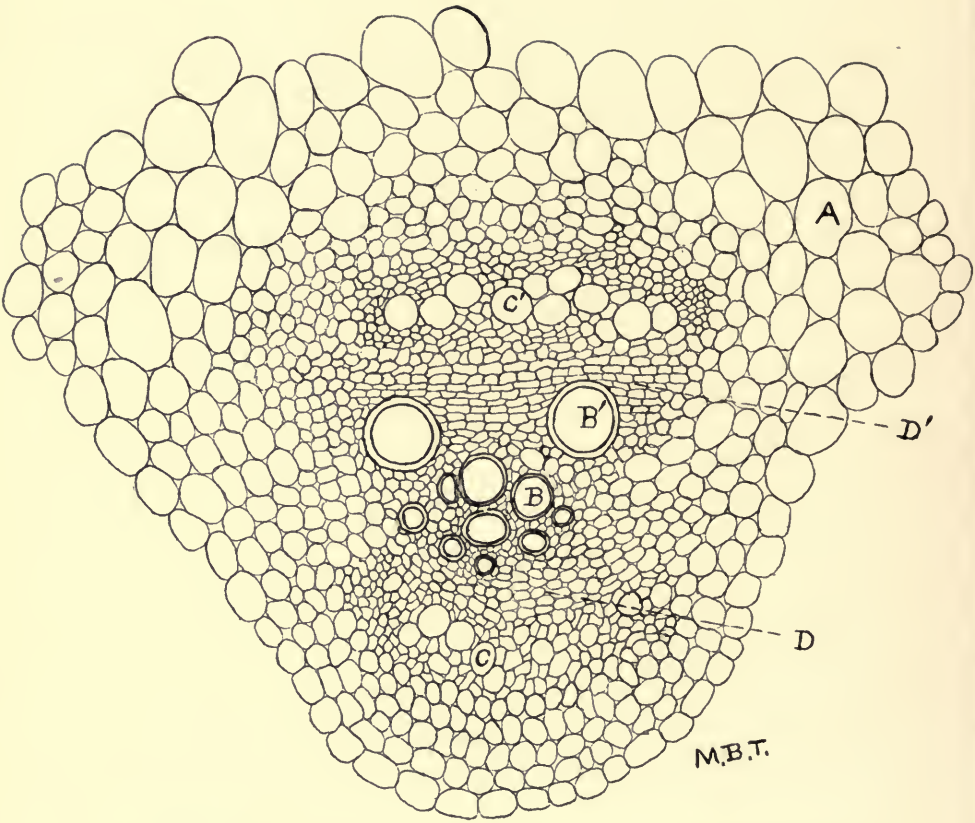

FIG. 20. Transection of a bicollateral fibro-vascular bundle from the stem of *Cucurbita Pepo*. A, Parenchyma cells of the fundamental system surrounding the bundle; B, B, large thick-walled vessels of the xylem; C, C, sieve-tubes of the phloem; D, D, thin-walled cells of the cambium. (x150.)

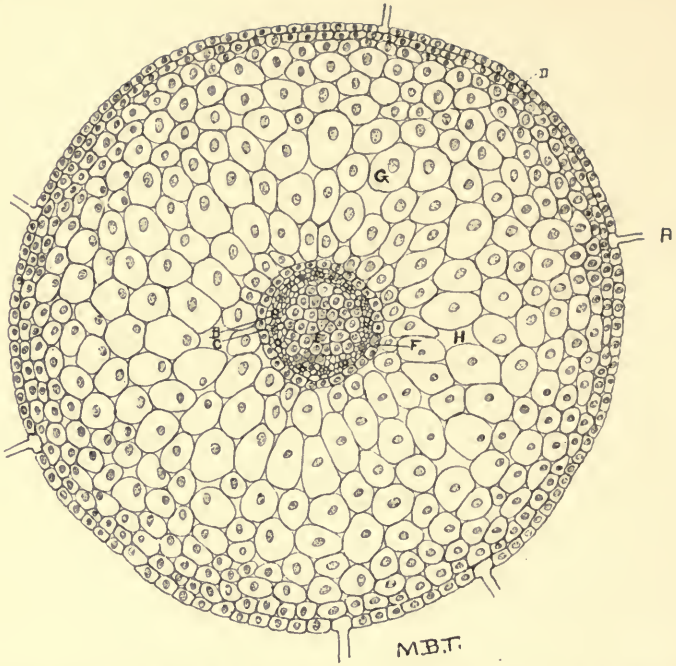

FIG. 21. Transection of the root of *Spiranthes cernua*. Showing the general structure of the root with the relations of the various parts. A, root hair; B, xylem of the radial bundle; C, phloem, alternating with the xylem areas; D, epidermis of the root; E, pith; F, bundle sheath or endodermis; G, cortex; H, intercellular space. (x50.)

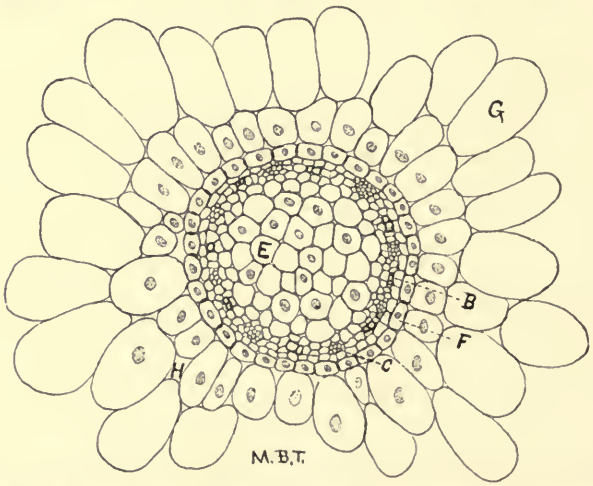

FIG. 22. The radial bundle in Fig. 21, more highly magnified. The lettering is the same in both figures. (x100.)

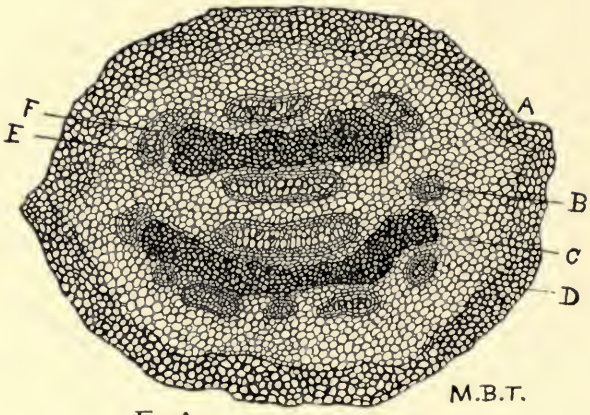

Fig. A.

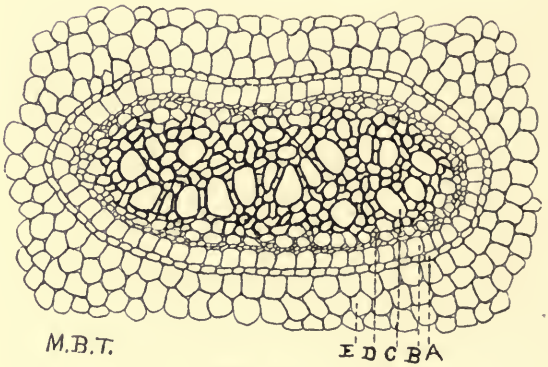

Fig. B.

Fig. 23. (Fig. A), Transection of the underground stem of *Pteris aquilina*. A, Epidermis; B, concentric fibro-vascular bundle; C, band of thick-walled brown sclerenchyma; D, thin-walled parenchyma of the fundamental system; E, xylem of the vascular bundle; F, phloem. (x17.)

(Fig. B). Enlarged concentric bundle from (Fig. A). A, bundle sheath; B, D, phloem parenchyma and sieve tissue; C, xylem of the bundle; E, parenchyma cells of the fundamental system forming the main mass of the stem and the "ground work" for the other tissues. (x100.)

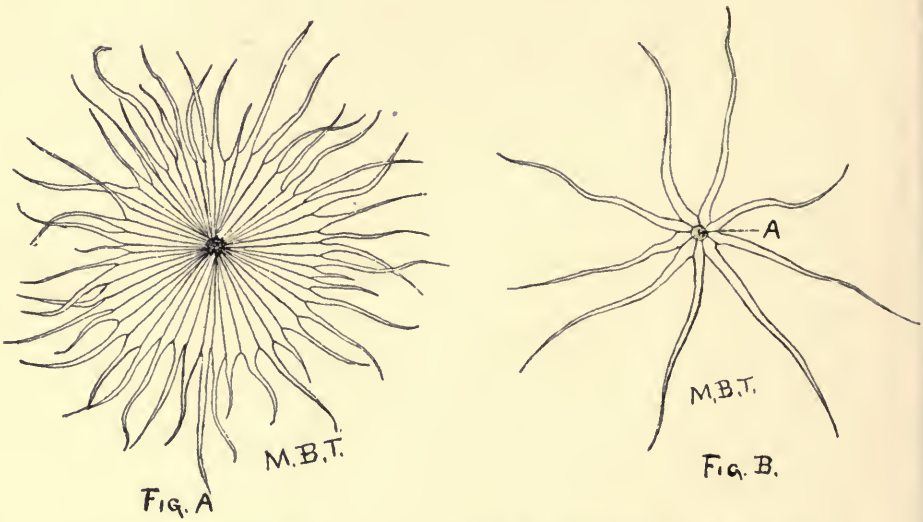

FIG. 24. Trichomes from the leaf of *Shepherdia Canadensis*. Fig. A, Peltate and Fig. B, stellate plant hairs. These are but variations of the same general type. A, Remains of the stalk. (x250.)

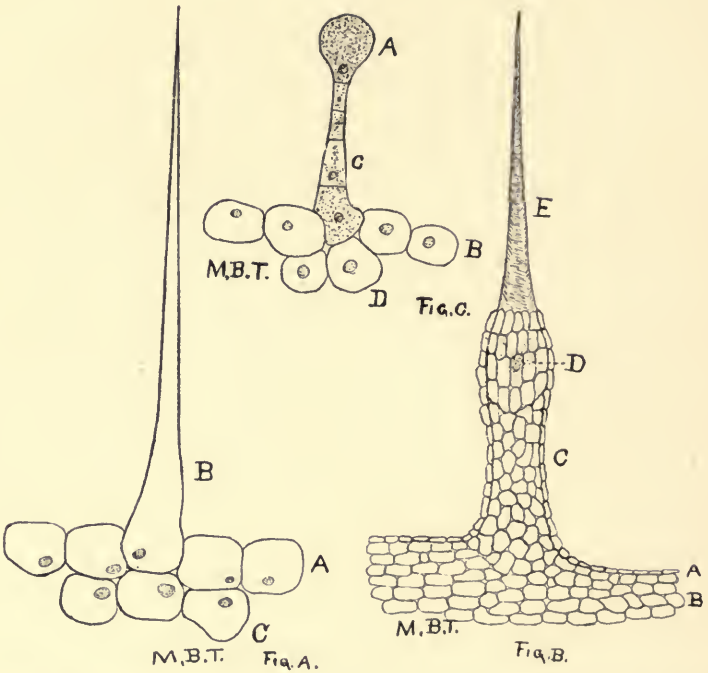

FIG. 25. Plant hairs (trichomes).

Fig. A. Needle like trichome from the leaf of *Geranium*, (*Pelargonium inquinans.*)

Fig. B. Stinging hair from the stem of *Urtica dioica.* A, epidermis of the stem; B, cortex; C, column of tissue supporting the hair; D, bulb of the hair and contained nucleus; E, slender tapering cell of the tip of the hair; the walls are strongly silicious and usually covered with fine transverse markings. (x50).

Fig. C. Glandular hair from the leaf of *Geranium.* A, Gland at the apex; B, epidermis of leaf; C, cells of the stalk; D, cortical parenchyma. (x200.)

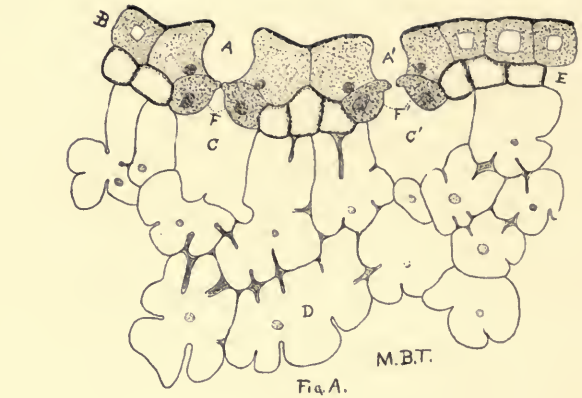

Fig. A.

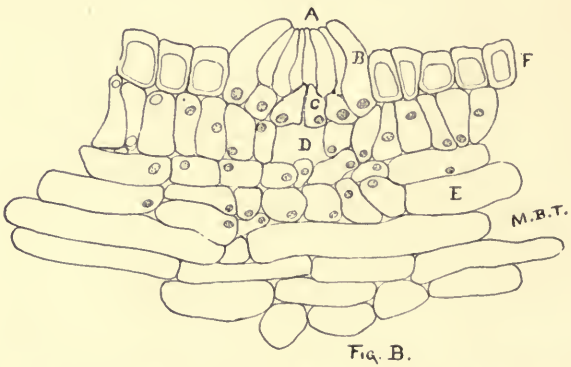

Fig. B.

FIG. 26. Fig. A. Transection of leaf, *Pinus sylvestris*. A, A, stomates; B, thickened epidermis; C, C, inter-cellular space beneath stomate; D, mesophyll of the leaf; E, thick-walled cells beneath the epidermis; F, guard cells of the stomate. (x250.)

Fig. B. Transection through the leaf of *Cycas revoluta* A, opening or pore of the stomate; B, modified epidermal cells; C, guard cells of the stomate; D, intercellular space into which the stomate opens; E, parenchyma cells of the mesophyll of the leaf; F, epidermal cells with strongly cutinized walls. (x250).

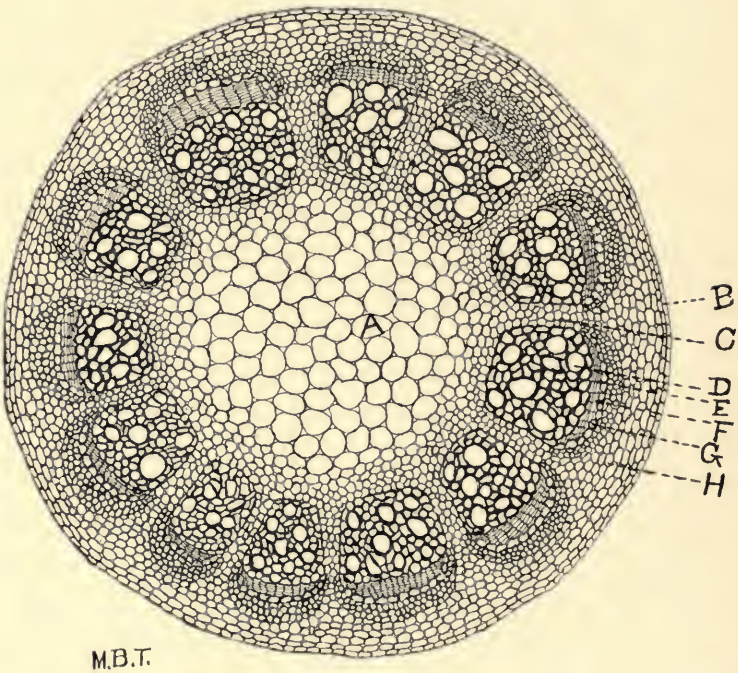

FIG. 27. Transection of the stem of a Dicot, *Menispermum Canadense*, (one year old). A, pith; B, epidermis; C, medullary ray; D, xylem; E, cambium; F, bast; G, sieve tubes; H, cortex. (x30.) Compare with stem of several years growth.

Plate XV.

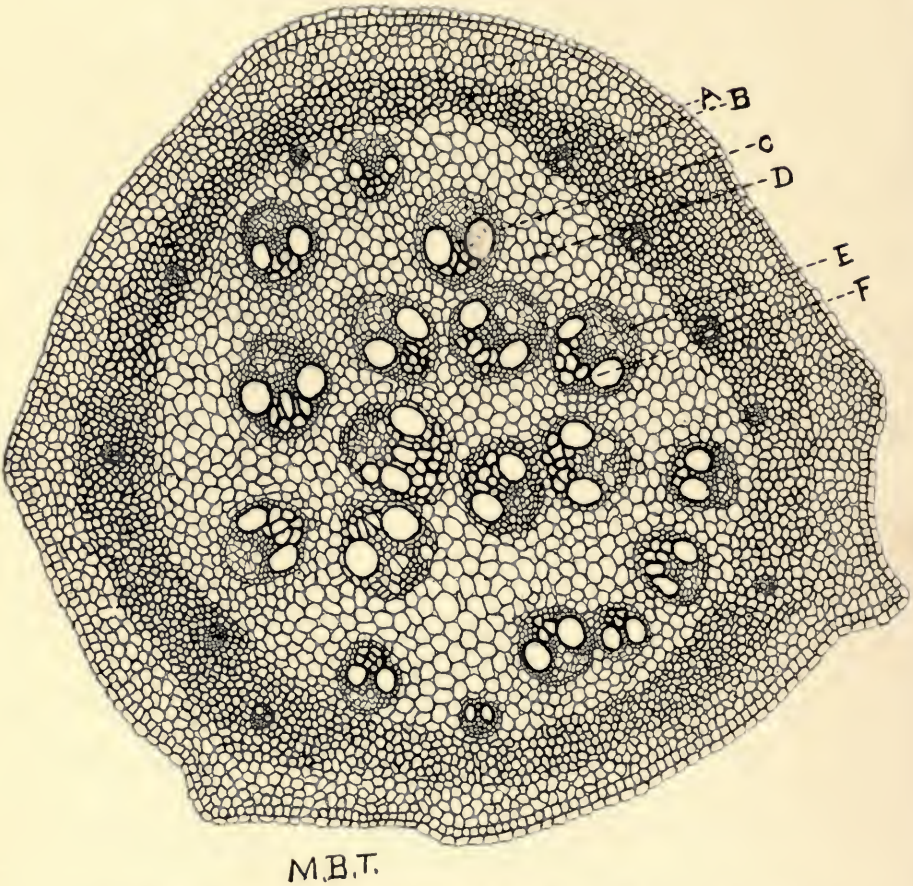

FIG. 28. Transection of the stem of a Monocot, *Smilax hispida*. A, epidermis; B, small closed fibro-vascular bundle, formed in the ring of meristematic tissue, where, by the development of new bundles, the stem is enabled to increase in size; C, mature vascular bundle; D, cortical parenchyma; E, phloem; F, xylem. (x80).