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PRACTICAL BOTANY

FOR MEDICAL, PHARMACEUTICAL AND
OTHER STUDENTS

By

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"A Textbook of Botany"; "Pocket Lens Plant Lore";
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"Origin and Development of Compositæ"; "Hydrogen-
ion Concentration in Plant Cells and Tissues," etc.

With 35 Illustrations



LONDON

J. & A. CHURCHILL

40, GLOUCESTER PLACE

PORTMAN SQUARE

1931

Printed in Great Britain.

PREFACE

THIS book is designed for use in conjunction with the author's "Textbook of Botany," as a practical supplement and general guide to the exercises in observation, experiment and investigation which usually accompany the study of general principles of which these exercises provide particular examples.

No attempt has been made to attain an exhaustive or inclusive course; but even now it is not expected that all the exercises will be carried out. Students and teachers vary in their requirements and interests, so that one or another aspect of the subject may be stressed and done in more detail. More advanced work in structure requires specialist books. More advanced studies in plant physiology should be definitely quantitative, and it is to be regretted that a book dealing systematically with such quantitative studies is not yet available. The extension of practical physiology into precise quantitative work is clearly outside the scope of a small volume dealing, as this one does, with general practical exercises.

The arrangement, following the Textbook chapter by chapter, is a consequence of the supplemental nature of the present book, and no departure from the usual sequence of practical work is intended. The writer has, however, proved that, by giving students card schedules with a numbered list of physiology experiments to be done (and initialled by the staff) at any available time, the average student can do far more practical plant physiology than is done in the usual crowded summer term. Plant physiology can then become an all-the-year-round subject. So many of the experiments have to be set aside for a varied period that a few minutes between lectures may be sufficient to get a long experiment into working order. Two such schedules are given in Appendix I., reproduced on a small scale.

The available literature has been used freely, especially

throughout the chapters on plant physiology, but the modifications introduced prevent particular acknowledgment of any definite experiment, except where that is done in the text.

Acknowledgments are gratefully tendered to Professor J. W. Heslop Harrison, F.R.S., for checking the problems set in Chapter XXVII.; to Dr. K. G. Emeléus and Mr. R. H. Sloane for the data concerning the energy transmissions of the colour screens, Chapter X.; to Messrs. Pastorelli & Rapkin for the block of Fig. 9; to Messrs. Robinson, Nelson & Co., 325 City Road, Manchester, and to Messrs. W. & J. George, of Birmingham, for the new pieces of apparatus and for the blocks of a number of figures; also to Miss M. J. Lynn and other colleagues and students for valuable assistance in checking the experiments and the proofs.

J. S.

BELFAST,

June, 1931.

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PRACTICAL BOTANY

PART I

CHAPTER I

INTRODUCTION

THE practical study of plants may be divided into three main types of exercise : **1. Observations**, which include the examination of the form and structure of plants or plant materials, together with records of these in the form of sketches, diagrams or careful detailed drawings. **2. Experiments**, which include the modification and control of the conditions under which the plant carries on living processes, together with records of the conditions and results of such experiments. **3. Investigations**, which usually include both observations and experiments, but which may also include the recording of facts concerning the conditions under which plants grow without any experimental modification or control of these conditions by the investigator, *e.g.* ecological survey.

In the following chapters the various exercises are numbered consecutively, but it will be found that the relative balance of observation, experiment and investigation is such that the work of the first session (in large print) is mainly observation, while the advanced work for second and third sessions (in smaller print) becomes more experimental, leading on to the investigations which should form an increasing proportion of the work of students as they progress towards a special Honours course and pass beyond the scope of this volume.

The value of practical botany for students who are not specialising in the subject lies primarily in the training which it involves in the accurate observation and correct recording of facts. These facts may appear to be of little consequence ; but it is perhaps better that the training should be carried out on materials where mistakes are not matters of life and death for the doctor's patient, or matters of survival or

ruin for the practical farmer. Such facts may be structural and recorded as drawings or manipulative and recorded as notes; in both cases the value of the observations is easily judged by the accuracy of the records.

Points to be Observed in Making Botanical Drawings

In most cases simple outline drawings are sufficient. The use of shading may give a pleasing artistic effect, but the outlines should give the details correctly, and no more is required. The use of shading tends towards the production of "impressions" rather than actual records of the facts as seen by careful observation.

It is especially important to show clearly not only the shape of an object, but also the correct proportions of its different parts. **DRAWINGS SHOULD BE MADE ON A LARGE SCALE.** This is necessary in order to show every part clearly, and the scale of enlargement or reduction should be noted, either by marking $\times 2$, or $\times \frac{1}{2}$, as the case may be, or by marking a short line near the drawing and stating the length which such a line represents, *e.g.* a line 1 cm. long might represent 1 km. on a map which is reduced, or 1 metre in a drawing of a tree, or 5 cm. in the drawing of a plant about 2 metres high, or 1 mm. in the drawing of a small fruit or seed, or $\frac{1}{10}$ mm. in a drawing of an object under the low power of the microscope, or 0.01 mm. if the high power of the microscope has been used (see Figs. 1, 2, 3, 4 and legends).

The Record-book should be kept clean and tidy. Prime factors in securing this are: (a) the proper spacing of the drawings, and (b) the use of only one side of the leaf for drawings, the other being reserved for descriptive notes. **Do not crowd your drawings.** All drawings should be fully named and labelled in the laboratory. The first-year student especially should annotate his drawings fully while the object is before him, and so secure an accuracy which is frequently lacking in drawings labelled later, using an imperfect knowledge and a very imperfect memory as guides to the details.

References

Since this book is designed for use in conjunction with the "Textbook of Botany" many references are given to

illustrations which are not repeated here. Such references are prefixed by "T" before the number of the figure, *e.g.* "Fig. T 764" will be found in the Textbook, while "Fig. 12" will be found in the present book. Page references are treated similarly.

A few simple contractions are used throughout, *e.g.*

T.S. = transverse section.

L.S. = longitudinal section.

V.S. = vertical section.

L.P. = low power of the microscope.

H.P. = high power of the microscope.

P-l.P.L. = "Pocket-lens Plant Lore." Small.
Churchill, London.

CHAPTER II

GENERAL INVESTIGATIONS

THE structure of plants varies from species to species, and there is a considerable probability that the physiology is as specific and varies from species to species in much the same way. In both structure and physiology there are general similarities and there are also differences in detail.

The student who has passed beyond his first year should, therefore, be encouraged to make a general investigation of one particular species. A thorough structural examination of the selected species will be valuable instruction both in the technique of microscope preparations and in the detailed variation of plant structure, for few species can be selected which are figured and described in detail in any book. The student is thus compelled at an early stage to make original observations and to make the acquaintance of facts which differ from textbook statements.

Further, very many of the usual experiments in plant physiology are carried out with prescribed plants. Second- and third-year students who are investigating a particular species should carry out these experiments using their selected species, whenever such material is available and suitable for the particular experiments. If the species chosen are common wild or garden plants with a foliage period covering a large part, if not the whole, of the year, it will be found that most experiments can be conducted using such material.

Species which have been treated in this way by the writer's students include—

Alopecurus pratensis, *Lolium perenne*, *Linum usitatissimum*, *Limnanthes Douglasii*, *Lamium purpureum*, *Solanum tuberosum*, *Bellis perennis*, *Tussilago farfara*, *Senecio vulgaris* and *Senecio jacobæa*; but the list could be very largely extended according to local conditions and requirements.

Professor E. J. Salisbury has given a very comprehensive schedule ¹ for the investigation of the biology of a flowering plant, which might well be followed in detail by third-year students. The scheme given below is partly based upon that schedule in so far as it is applicable to the species considered; the structural

¹ *Journal of Botany*, Vol. LXVI, p. 48, 1928. See also Small's "Textbook of Botany," Chapter II, for "The Dandelion."

investigation and the use of the species for plant physiology experiments have been found to be a very useful training and a stimulus for students of general botany and of agriculture. The principle of specific investigations might profitably be extended to non-flowering plants where material is available in sufficient abundance.

SPECIFIC INVESTIGATIONS

Each student is required to make an investigation of one species, according to the list posted on the notice-board at the beginning of each session. The reports on these investigations must be submitted to the Professor on or before June 10th.

These investigations should include as far as possible the following points :—

A. An illustrated anatomical account of the stem, leaf and root of the plant, as ascertained using both transverse and longitudinal sections; particular attention should be given to the course of leaf-traces, the number of vein-islets per square centimetre, the number and distribution of stomata, the form of any accessory cells of the stomata when present, the transition from stem to root and any features mentioned under C.

B. An illustrated account of the structure of the flower, including a floral formula, floral diagram, median longitudinal elevation and large-scale annotated drawings of any parts which have a special biological significance (see C).

C. A detailed account of the biology of the selected species, including: Characters of the natural soil, such as water content, organic content, reaction, etc. (T. pp. 397 *sqq.*): light or shade type: biological type; duration of plant, mode of perennation and vegetative multiplication, duration of leaves: phenology of first leaf, first flower, seed ripening, leaf-fall: root system; type, volume, depth, special features; shoot system; height; leaves; position, glands, transpiration arrangements, special anatomy: reproduction; pollination, self-sterility, etc., mechanism, agents, special features; seed output, size and weight of seed, dispersal, natural period of germination and percentage germination: survival values, including hardiness, protection, recovery, and diseases or parasites peculiar to the species.

D. A summary account, extracted from the student's own notebook, of all the results of physiological experiments carried out upon this particular species.

If such an investigation be combined with the study of all available literature concerning his selected species, the student will have the intellectual satisfaction of knowing a great deal about at least one plant, as well as the practical satisfaction of knowing how to proceed with a general survey of what a plant is and what it does.

CHAPTER III

SEEDS

N.B.—Read Chapter I carefully before commencing work.

1. Examine seeds of the **Broad Bean** (*Vicia faba*) of which the bean pod is the fruit (cp. Fig. T 764). Make drawings to show (a) **hilum** (scar of seed-stalk), (b) **micropyle**,¹ (c) **radicle** lying below the seed-coat. Remove the seed-coat (*testa*). Examine the embryo plant; it consists of (a) **plumule** or young stem, (b) **cotyledons** or seed-leaves, (c) **radicle** or young root, the tip of which lies in a pocket of the testa near the micropyle. Open the cotyledons apart gently, make a drawing of the embryo-plant at least **THREE** times natural size, and label all the parts (Fig. T 2).

2. Examine and draw a grain of **Indian Corn or Maize** (*Zea mais*). This is a one-seeded fruit with the seed-coat and ovary wall fused. Note the lateral whitish portion showing the position of the embryo, with a slight central ridge. Cut the grain in half along the middle of this ridge, taking care to use a sharp knife and to place the knife-edge exactly in the middle of the central ridge. The cut surfaces will each show a median longitudinal section of the grain. Note the parts exposed and make a drawing of the section at least **FOUR** inches in length; label all the parts to show (a) the **endosperm**, which is usually translucent and horny towards the outside, white and granular towards the embryo; (b) the embryo, with a single cotyledon (**scutellum**) close to the endosperm, with **plumule** and **radicle** towards the outside. The plumule and the radicle are each enclosed by a sheath, and when the fruit is cut in this way these organs can be lifted with a needle point to show the enclosing sheaths, (Fig. T 3).

3. Examine and draw a single fruit of **Sunflower**

¹ These seeds have been soaked overnight in water. If one seed be carefully dried and then gently squeezed, water can be seen to ooze out of a small hole at one end of the hilum. This hole is the micropyle.

(*Helianthus annuus*). Split the hard ovary-wall open ; pressure on the edge of the flattened fruit separates the two valves which together form the fruit-wall. Note the single seed which is thus exposed. Remove the very thin soft seed-coat and examine the embryo, which consists of two cotyledons, a small downwardly directed radicle apparently attached at the top to the cotyledons, and a very minute stem apex lying between the cotyledons. The seed-leaves,

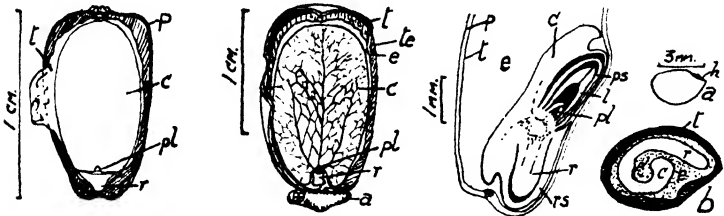


Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

FIG. 1. — Dissection of Sunflower fruit : *p*. pericarp, *t*. seed-coat, *c*. one of the two cotyledons, *pl*. stem apex, *r*. radicle.

FIG. 2. — Dissection of Castor bean : *t*. brittle testa, *te*. thin tegmen, *e*. oily endosperm, showing cut surface shaded, *c*. one of the two membranous cotyledons, showing distinct venation, *pl*. stem apex, *r*. radicle, *a*. aril or caruncle.

FIG. 3. — Lower part of grain of Wheat : *p*. pericarp, *t*. testa — not distinguishable with a pocket-lens, *e*. endosperm, *c*. cotyledon, *ps*. plumule sheath, *l*. first foliage leaf, *pl*. remainder of plumule, *r*. radicle, *rs*. radicle sheath.

FIG. 4. — Onion seed : A. flat side of seed, *h*. hilum, x4 ; B. seed cut open to show embryo with *c*. single cotyledon, *r*. radicle, *pl*. position of stem apex which is inside the cotyledonary tube (cp. Fig. T 37B), *e*. endosperm, *t*. seed-coat.

here as in the broad bean, occupy the bulk of the space inside the seed-coat. Remove one cotyledon and make a drawing at least 2 inches long of the remaining parts of embryo (Fig. 1, cp. Fig. T 20). The Almond has a proportionately larger plumule.

4. Examine and draw a single **Castor Bean**, the seed of *Ricinus communis*. Note the smooth, hard, mottled surface of the outer seed-coat and the caruncle or micropylar aril. The ridge which may be seen along one face of the seed corresponds with the raphe of the ovule (Fig. T 520). Crack

carefully and remove the outer seed-coat (**testa**), and observe that the contents are surrounded by a very thin inner seed-coat (**tegmen**). Scrape off the inner seed-coat and cut open the endosperm carefully with the point only of a penknife. When cut along one edge the endosperm opens easily, and then the thin membranous cotyledons can be seen closely adpressed to the inner surfaces of the cavity in the endosperm. This cavity is best seen by cutting a whole seed transversely (see Fig. T 6). Draw one-half of the contents of the seed-coat to show (a) the veined cotyledon on the surface of the oily endosperm, (b) the distinct radicle, (c) the very minute stem apex (Fig. 2 and Fig. T 6).

5. Examine a black **peppercorn** (the fruit of *Piper nigrum*) carefully. Note the two scars at the poles of the rounded wrinkled grains. One scar indicates the base or point of attachment of the sessile fruit and the other is formed by the remains of the sessile stigmas. Cut the grain in half with a sharp knife so that the cut passes through both scars. Examine the longitudinal section as shown on the surface of one half. Make a drawing at least 1 inch in diameter to show (a) the black fruit wall, (b) the brown seed-coat, (c) the hollow sphere of perisperm, (d) the small hood of endosperm, (e) the minute embryo lying within the endospermic hood (Fig. T 4, B).

6. Examine the grain (fruit) of **Wheat** (*Triticum sativum*), **Oat** (*Avena sativa*) or **Barley** (*Hordeum vulgare*). Cut one grain in half longitudinally along the median plane and make a drawing at least 2 inches long to show the parts (cp. Fig. T 9 and Exercise 2). The other cereal grains (fruits) do not differ essentially from the Maize, but the embryo is placed nearer to the base in the grains of wheat, etc. (Fig. 3).

7. Using a good pocket-lens, examine a number of the seeds of the **Onion** (*Allium cepa*) carefully. Note the usual presence of one larger flattish oval side and the hilum at one end. Choose a seed with a distinctly flat side, lay that upon the bench and cut the top off the seed, parallel to the flat side, with a sharp knife. Examine the cut surface with a pocket-lens. The embryo should be seen lying embedded in the semi-translucent endosperm, the whole surrounded by the black seed-coat. The cotyledon is terminal, the stem apex lateral and the radicle coiled round, so that it is directed towards the hilum, where the micropyle also is

situated, although it cannot be seen normally with a pocket-lens (Fig. 4). N.B.—There is some variation, and several seeds may be cut before a typical view of the embryo is obtained.

These seven seeds include four dicotyledons (broad bean, sunflower, castor bean, pepper) and three monocotyledons (maize, wheat or other small cereal, onion). These examples illustrate the main varieties of seeds—namely, dicotyledonous, with two seed-leaves, with no endosperm, as bean and sunflower; with endosperm and two seed-coats, as castor bean; with endosperm and perisperm, as pepper-corn: monocotyledonous, all with endosperm, with lateral embryo, as maize and other cereals, and with embedded embryo as onion.

8. Medical and pharmaceutical students should also examine and draw the external features of a selection of distinctive seeds used in medicine, such as stavesacre, calabar bean, fenugreek, strophanthus, nux vomica, stramonium, henbane, grains of Paradise and areca seeds.

See "Materia Medica," by H. G. Greenish. Churchill, London.

9. Agricultural students should also examine and draw the external features of distinctive seeds of interest to farmers, such as those of crop plants and of common weeds. Distinctive features are shown by the following seeds or small one-seeded fruits of crop plants: hop, buckwheat, flax, clovers, sainfoin, potato, various grasses; and by the following common weed seeds: docks, knotgrass, persicaria, chickweed,ampions, white goosefoot, creeping and acrid buttercups, salad burnet, gorse, dove's-foot and cut-leaved cranesbills, violets, dodders, plantains, yellow-rattle, self-heal, and a number of Compositæ fruits such as chicory, milfoil, corn chamomile, ox-eye daisy, sow-thistle, dandelion, nipplewort, knapweed and field thistle.

See "Agricultural Botany," by J. Percival, Duckworth, London; and the excellent "Photomicrographs of Grass, Clover, Forage Plant and Weed Seeds," issued privately by Messrs. James Hunter, Ltd., Chester, England; also "Impurities in Agricultural Seed," Parkinson and Smith, Headley Bros., Ashford.

10. More advanced students, especially during their "specific investigations," should study the structural details of seeds, particularly in relation to the characteristic family features shown by seed-coat, perisperm, endosperm, presence or absence of starch, development of cork, etc. See "Anatomie der Angiospermen-Samen," by F. Netolitzky, Borntraeger, Berlin. Refer especially to p. 40 and the table on pp. 309-321.

CHAPTER IV

GERMINATION AND SEEDLINGS

11. Examine and draw seedlings of the **Broad Bean** (*Dicotyledon*) in various stages of development, such as : (a) emergence of the radicle through the ruptured seed-coat ; (b) elongation of the radicle and emergence of the plumule, which is withdrawn from between the cotyledons by the elongation of that part of the stem (the **epicotyl**) which lies above the cotyledons and below the first foliage leaf ; (c) the straightening of the plumule with unfolding of the foliage leaves and formation of lateral rootlets on the primary root (Fig. T 31). The region of growth in the stem of the bean seedling is above the insertion of the cotyledons, and therefore the cotyledons remain below ground. This gives **Hypogeal Germination**.

12. Examine and draw seedlings of the **Sunflower**. Germination is similar to that of the broad bean except that the region of growth in the stem is below the insertion of the cotyledons, *i.e.*, in the **hypocotyl**, and the seed-leaves are therefore carried above-ground, giving **Epigeal Germination** (*cp.* Fig. T 36, B and C).

13. Examine and draw stages in the germination of **Maize** (*Monocotyledon*), such as : (a) first emergence of radicle, through the enlarged radicle sheath which now forms a collar (**coleorhiza**) around the top of the young root ; (b) emergence of the plumule-sheath, which is split open from the inside by the first foliage leaf after the elongated sheath (now called the **coleoptile**) has grown to 1 or 2 inches in length ; this stage usually includes the elongation of adventitious rootlets which burst through the upper part of the coleorhiza ; (c) foliage leaf unfolded, surrounded at the base by the coleoptile, and numerous adventitious roots forming the permanent root-system (*cp.* Fig. T 32). The single cotyledon remains below ground, acting as a haustorium, giving **Hypogeal Germination**.

14. Examine and draw stages in the germination of the

Onion, or of the **Date** (see Fig. T 37). In both cases there is a cotyledonary tube which elongates, carrying the radicle downwards and leaving the tip of the cotyledon closely applied to the endosperm within the seed-coat, stage (a). In both cases this tip remains below ground in firm soil, or carries the seed above ground in loose soil, stage (b). In the former event the cotyledonary tube becomes strongly bent into a loop before the first foliage leaf (onion) or the plumule sheath (date) emerges from a slit in the tube, stage (c). The type of germination is technically hypogeal because the cotyledon does not come above ground under natural conditions, nor does it turn green and become an assimilating organ. Date stones or seeds germinate readily if kept submerged in water in an incubator at 50°-60° C.

15. The first-year student should examine carefully and take illustrated notes upon the experiments 16 to 20 set up as demonstration experiments for his observation.

16. Water and Oxygen. The effects of water and air (oxygen) on germination are clearly shown by "the Three Bean Experiment" (see Fig. T 31). The beans should be used in the air-dry or ordinary condition, in which it is possible to pierce the cotyledons with *strong* pins and impale three at different levels. The water should be boiled in the beaker which is to be used, and left to cool without any disturbance. The results should then be as figured and described (Textbook, p. 20).

17. Grains of barley or other cereal should be treated as in Ex. 16. The results are similar.

18. Temperature. Prepare a freezing mixture of common salt and broken ice. Prepare four small beakers by lining the bottom of each with wet cotton-wool; place twenty grains of barley or other cereal on the wool in each beaker. Arrange each smaller beaker inside another larger beaker which is at least 2 inches more in diameter. Place the freezing mixture inside one of the large beakers, around the outside of the smaller beaker; pour as much cold water into the second large beaker as you can without floating the small beaker; pour hot water in the same way into the third large beaker. Set three of the experiments aside in a well-lighted place and continue to take notes on the results at intervals for at least two weeks, replacing the cold water in the third beaker with hot water on each occasion after you

have taken your notes. The fourth pair of beakers is used in Ex. 19.

Mustard or cress may be used instead of a cereal. Germination in all cases should not take place in the first beaker, and should be stimulated at first in the third beaker, but after a time there may be little or no difference between the second or normal seedlings and the third or heat-stimulated seedlings.

19. Light. Pour cold water into the fourth large beaker as for Ex. 18, second set. Place this beaker, with the smaller one containing twenty seeds on wet cotton-wool, in a dark cupboard. Keep the temperature of the water-bath as near as possible to that of the second set, Ex. 18, and take notes at intervals for at least two weeks. The pale colour and other abnormal features of seedlings raised in the dark are known collectively as **etiolation** and show that light is necessary for *normal seedling development*.

20. While light is necessary for normal development it may inhibit or stimulate actual germination. Procure seeds of one or more of the following species; place at least ten seeds in each of two "germinators" (see Ex. 22), setting one lot aside in a well-lighted place and the other in a dark well-aired cupboard. Note the temperatures around each germinator and take notes at intervals for at least three weeks.

Species. Most meadow-grasses (*Poa* spp.), which require light for normal germination; tobacco varieties (*Solanum tabacum*) which germinate more readily in light; columbines, particularly *Aquilegia atrata*, and purple loosestrife (*Lythrum salicaria*) which show very little germination in the dark; tomato varieties (*Solanum lycopersicum*), love-lies-bleeding (*Amaranthus atropurpureus*), and to some extent the large field speedwell (*Veronica buxbaumii* or *tournefortii*), which all germinate more readily in the dark and show little or no germination in the light.

Note. Each seed which puts out a radicle should not be removed, but its germination should be recorded. Records of germination should be numerical in the first place, followed by notes upon subsequent developments under the different conditions. See Ex. 22 for germinators.

21. More advanced students should dissect and make drawings of some or all of the following interesting seeds and seedlings. **Mustard** (*Sinapis*), epigeal germination with each cotyledon two-

lobed. **Cress** (*Nasturtium*), epigeal germination with each cotyledon three-lobed.

Ash (*Fraxinus*), one side of the samara wall should be dissected off and the elongated seed-stalk noted, together with the two lobes of oily endosperm enclosing an embryo with two small cotyledons and a minute stem apex. The seedlings are common under the tree.

Sycamore (*Acer*), can be dissected in the same way, when the embryo will be found to have two quite green seed-leaves coiled into a spiral with the radicle towards the outside. These seeds germinate and develop exceptionally well for a time in the dark. The seedlings are also common in the neighbourhood of the parent tree.

Beech (*Fagus*), has a one-seeded nut with no endosperm and the small seed should be compared with the seedlings in which the two cotyledons become large, rather leathery, oval green leaves. These developed seed-leaves carry on all the carbon assimilation of the seedling for several weeks. The seedlings are very common in open beech-woods. See also "Pocket-lens Plant Lore."

Pine (*Pinus*), shows winged seeds which germinate readily if kept in slightly moist sand in the dark at a temperature of 15°-20° C. The cotyledons, which vary in number from five to eight, are apt to carry the seed with its endosperm above ground under these conditions, but the seed-leaves soon spread out and become dark green even in the absence of light. The seeds take from three to five weeks to develop into seedlings.

22. Germination Capacity. In technical seed-testing purity, i.e. freedom from seeds of other plants and from inert matter, is important, but the botanical student is concerned mainly with the viability of seeds. The proportion of dead seeds increases rapidly in many species after the first year of storage.

Willow seeds can be used to demonstrate this falling-off of viability. If seeds be collected from newly ripe catkins and tested at daily intervals, as described below, the germination capacity will be seen to fall rapidly after the first few days. For this test a separate germinator must be arranged each morning for ten days and supplied with fifty willow seeds. Coltsfoot seeds show a similar rapid decrease of viability.

The percentage of live seeds is of considerable economic importance and seed-testing has a special technique which is largely conventional, or empirical. The student should, however, carry out at least one exercise to determine the germination capacity of a particular sample of seeds.

(a) Prepare a "germinator" by selecting a Petri capsule about 12 cm. diameter; fold a piece of wet filter paper over a glass slip about 20 cm. by 6 cm.; half fill the lower Petri half-capsule with water; place the glass and paper across it so that the free ends of paper dip into the water; and cover with the upper half of the

Petri dish (see Fig. 5). Upon the square of paper which is thus kept moist the seeds to be tested should be laid out carefully in ten rows of ten each for small seeds, with others in proportional numbers; less than fifty will give a very unreliable result. Place the germinator near a radiator or in some other place where it will be more or less protected from low night temperatures. Keep the water fresh by renewal at intervals.

The number of seeds which show a radicle with healthy root-hairs should be counted and noted each day. This counting is more easily done if all the seeds which have germinated each day are removed from the germinator. After two weeks, or in the case of grasses three weeks, the total number of germinations should be noted. If 100 seeds are used the percentage germination or germination capacity is obtained directly, otherwise it is

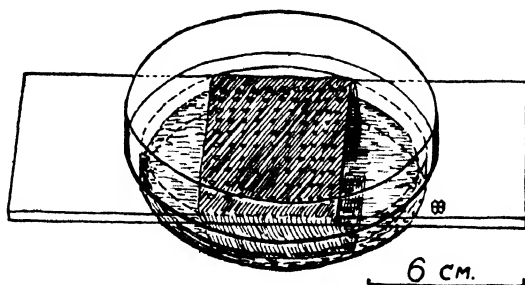


FIG. 5.—A Simple Germinator.

calculated on the basis of the number of seeds used. It should be noted that, if the germination capacity be 80 per cent. and there be 10 per cent. by weight of weed seeds, the germination value or "real value" is only 90 per cent. of 80 per cent., *i.e.* 0.9 of 80 per cent. or 72 per cent.

(b) Using any available small seeds, take 60 and divide them into three equal lots, A, B, and C. Place lot A in a dry test-tube, immerse lots B and C each in half a test-tube of water for twenty-four hours. Heat lots A and C in a water-bath or water-oven for two hours at 80° C. Determine the percentage germination of each of the three lots separately; A should be full capacity, B slightly less and C minimal or zero. The effects of temperature and immersion in water each depend upon the other conditions; dry seeds survive high temperatures more readily than do moistened seeds, and some seeds recover from the effects of dry heat.

CHAPTER V

THE MICROSCOPE AND THE CELL

BEFORE the student proceeds to further studies of the plant and how it works he must have some acquaintance with (a) the microscope and (b) the microscopic units of which the plant is built.

THE MICROSCOPE

23. I. Parts of Microscope. Examine the microscope provided and recognise the following parts : foot, body-tube, coarse and fine adjustments, stage, diaphragm, mirror, nose-piece, objectives (low and high power), eye-piece, draw-tube.

II. Mounting. (a) Most objects are examined by transmitted (not reflected) light and must, therefore, be thin and transparent.

(b) Place the object in a very small drop of mounting liquid (*e.g.* water, glycerine, etc.), in the centre of a clean glass slide.

(c) Carefully lower, by aid of a mounted needle, a clean cover-slip on the surface of the liquid. It is very important to use the proper amount of liquid, *i.e.* just enough to reach the edge of the cover-slip, and no more ; otherwise the liquid will often get on the top of the glass, and so to the objective of the microscope.

N.B.—Never use the microscope on an object without a cover-slip, unless this is expressly directed, as in **Ex. 30.**

(d) Staining (with special reagents) may be done either on a slide or in watch-glasses before transferring to the slide.

(e) One mounting or staining fluid may be replaced by another by irrigation without removing the cover-slip. Place a small drop of the second fluid on the slide at the edge of the cover-slip and draw through by blotting paper applied on the opposite side.

III. Use of Microscope. (a) See that the eye-piece and objectives are clean.

(b) Adjust the mirror so that sufficient light reaches the eye-piece.

(c) Always find the object with the low power first, focussing with coarse adjustment; and then move the object into the centre of the circular field of vision.

(d) If greater magnification be required, see that the draw-tube is pushed in, RAISE THE BODY-TUBE with the coarse adjustment for $\frac{1}{2}$ inch; swing round the high power; then, with the eye at the level of the stage, slowly lower the body-tube until the high power objective almost touches the cover-slip; and focus UPWARDS ALWAYS with the fine adjustment, keeping the eye looking into the EYE-PIECE while doing so.

(e) Never apply force to either coarse or fine adjustment when focussing. If anything appears to be wrong with a microscope call the attention of a demonstrator or attendant at once.

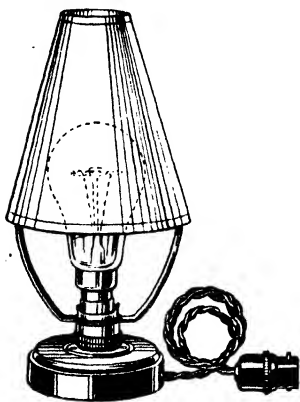


FIG. 6.—Small's Microscope and Bench Lamp.

IV. Illumination. (a) In examining delicate objects, the finer details of structure can often be seen only if part of the light be cut off by means of the diaphragm underneath the stage.

(b) A north window gives the most suitable daylight for microscopic illumination.

(c) Artificial lights should be scientifically shaded as in the lamp illustrated (Fig. 6).

THE CELL

24. The free mature plant cell—as an essentially spherical body limited in size by a cellulose wall and containing a thin film of cytoplasm, in which occur a nucleus and possibly other bodies, and which constitutes a large vesicle enclosing vacuolar sap—can be seen most readily in the fleshy fruits. Take a very small portion of the soft fleshy part of an apple or tomato or snowberry or similar fruit. Mount in water as

described above in 23, II (b) (c), squashing the tissue gently with the cover-slip.

The spherical cells are easily distinguished under low power. Choose a few very distinct cells and move the slide so that these are in the centre of the field. Change to high power, note carefully and draw (a) the cellulose wall, (b) the granular cytoplasm, (c) the highly refractive nucleus which changes in appearance with slight alterations in the fine adjustment of the microscope, (d) the transparent vacuolar sap, and (e) any other cell contents which may be present, e.g. small brownish-red chromoplasts in the tomato.

25. The plant cell as the mature unit built up into a tissue, with its spherical extension limited by the pressure of neighbouring cells, is most readily seen in epidermal tissues which can be easily stripped from the leaves of many monocotyledons.

Strip the inner side of an onion-bulb scale, or the leaf of the iris, hyacinth or daffodil; mount and examine a small portion. Draw carefully, using H.P., at least one cell together with all the cells surrounding the first one (*cp.* Fig. T 261).

26. The plant cell as a uniform unit, which at first contains only cytoplasm and nucleus, and later becomes vacuolated and differentiated to form different tissues, is most readily seen in prepared longitudinal sections of root-tips. Examine the prepared section supplied, under the low power. Note the dense small cells just above the root-cap; these are the youngest cells and are all very similar, with nuclei, but no vacuole. Observe the progressive vacuolation as you pass from the first region to those regions which are higher up the root. Observe also the gradual change from uniform structure and size to a variation giving an outer layer, and conducting strands, with relatively uniform tissue as a ground-work.

Draw carefully, using the H.P., a few cells from each region, and label your drawings to show the relative positions of the cells within the root-tip (*cp.* Fig. T 71).

27. Chloroplasts. Amongst all the various cell-contents, there are no more important bodies than the green plastids, which are the centres of sugar and starch formation.

(a) Mount the leaf of a moss in water; note and draw a few of the cells with their contents, mainly chloroplasts.

(b) Squash the succulent leaf of a small stonecrop (*Sedum*

spp.) on a slide with the point of a knife, mount under a cover-slip with a little water. Examine under low power. Choose one of the oval cells, which should lie free in the water, and examine under high power. Draw this cell in detail as an example of the complete, free and mature green plant cell (*cp.* Fig. T 79).

Advanced students should study the variation in chloroplasts and chlorenchyma, using various algae such as *Zygnema*, *Mougeotia*, *Spirogyra* and *Cladophora*, etc. (see Chapter XXXIII), *Anthoceros* and various mosses (see Chapter XXXVII), and the leaves of *Selaginella*, *Pinus*, *Sambucus*, *Paeonia*, *Anemone*, etc.

28. Starch Grains and Starch Reaction.

(a) Scrape off (do not cut) a small portion from the freshly cut surface of a potato. Mount in a small drop of water; cover with a cover-slip. Examine under L.P. and note the numerous starch grains. Choose a few large grains clear of the tissue; place these in the centre of the field and change to the high power of the microscope. Reduce the illumination, if necessary, by means of the diaphragm or stop below the stage. Observe and draw a few grains to show the **general oval shape**, the **hilum** or dark spot towards one end and the irregularly **concentric striæ**, some of which are more conspicuous than others (Fig. T 58). Irrigate your preparation (see 23, II, e) with solution of iodine and note the dark blue colour produced. This is a general test for starch.

(b) Test for starch with iodine solution placed on the cut surface of the bean cotyledon (*cp.* Fig. T 52) and of the maize endosperm (*cp.* Fig. T 3, c).

29. Leucoplasts, Chloroplasts and Chromoplasts.

(a) Starch grains are produced by chloroplasts or by similar plastids which are white (leucoplasts). Cut a thin section of *Pellionia* stem and examine the outer part of the cortex carefully. Observe and draw some of the transitions from chloroplasts to leucoplasts which are shown (Fig. T 64).

(b) Yellow chromoplasts will be seen in the hypodermal cells of the yellow or red parts of the tulip perianth. Red rod-shaped chromoplasts are easily found in the coloured fleshy parts of the tomato fruit.

30. (a) **Anthocyan.** Examine thin sections of beetroot and note the colouring matter, which flows out when the cells are broken open by pressure on the cover-slip. Pelar-

gonium petals and red tulip flowers show similarly coloured sap in the epidermal cells.

(b) **Free Organic Acids.** Cut out a fairly large eye-bud from a potato; take a number of longitudinal sections of this bud and place them in distilled water. Mount one section in distilled water and examine it carefully, paying particular attention to the presence and distribution of cells towards the outside which may contain pink colouring matter (anthocyan). Mount another section, upon a perfectly clean slide and *without* a cover-slip, in a large drop of balanced diethyl red indicator solution.¹ Observe with the naked eye the appearance, within five minutes, of a red colour in the peripheral regions of the cortex. Examine this section under L.P. and compare it with the control in distilled water. The red coloration indicates the presence of free organic acids which occur in the sap of these cells, as well as in the epidermis of the bud, giving an acidity at least greater than pH 5.6, the change point of diethyl red. Young rootlets from sprouting potatoes will also show this phenomenon. Note that some other cells in the section remain yellow if no cover-slip be used. Cover the section with a cover-slip and observe the gradual spreading of the red colour over the whole of the section. This acidity is caused by the accumulation of carbon dioxide below the cover-slip.

Make a diagram to show the distribution of the cells containing free acid. Other indicators may be used (see Appendix II).

31. Calcium Oxalate Crystals. These occur in various forms (see Figs. T 47-50).

(a) Cut a thin longitudinal section of the lower part of the dock-leaf stalk (*Rumex*) or a transverse section of the leaf-base of horse-chestnut (*Aesculus*). Mount in alcohol and add water. Examine under L.P.; note the spherocrystal clusters throughout the section. Draw one cluster in detail under H.P. (*cp.* Fig. T 48). Similar crystals are common, but not usually numerous, occurring in the wide part of the medullary rays in the bark of lime (*Tilia*), see Fig. T 398, in the cortex of beet (*Beta*) stem and root, in the endosperm of fennel fruits, etc.

(b) Cut a number of *longitudinal* shavings from cascara bark. Place them in alcohol in a watch-glass. Select a

¹ See Appendix II. .

few which are thinner at one end. Mount these in water under a cover-slip. Examine the thin ends under L.P. for projecting ends of thick-walled bast fibres. Examine these parts carefully under H.P. and observe the almost rectangular refractive crystals (calcium oxalate) which occur in rows near these fibres. Each crystal occupies nearly the whole of one parenchymatous cell. Draw a few crystals under H.P. (*cp.* Fig. T 47). Similar crystals are easily found in longitudinal sections of B.P. Quassia wood (*Picraena excelsa*).

A few similar crystals may be found in the bark of old stems of soap bark (*Quillaia*), buckthorn (*Rhamnus frangula*), elm (*Ulmus*) and alder (*Alnus*), also near the midrib of senna leaves, in sandalwood and in the sclereids of calumba root. Longitudinal sections show these better, since the squarish refractive crystals are not then so easily confused with cross-sections of fibres. The high power is required for these small crystals. Similar crystals occur just below the epidermis in orange or lemon peel.

(c) Open the base of an old leaf of *Kniphofia*, and scrape off a small quantity of the mucilaginous material which lies between the upper and lower surfaces of the leaf. Mount this in water; examine and draw *under low power* one of the large bundles of needle crystals (raphides). Squash the preparation gently with the cover-slip and draw a few of the isolated crystals which are freed by the bursting of the cell-wall and mucilaginous sheath of the crystal bundle (*cp.* Figs. T 49-50). These crystals are exceptionally large,¹ as are some of those in B.P. squill bulb-scales. Similar but fewer and smaller raphides may be found in the flower stems or leaves of hyacinth, arum, onion and other monocotyledons, including also the leaves which crown the fruit of the pineapple, the bulb-scales of squill and the root of ipecacuanha in longitudinal sections cleared in chloral hydrate.

(d) **Tests for Calcium Oxalate.** Iodine stains brown the rhomboidal crystals of protein which resemble the second type of calcium oxalate crystals, but does *not* stain calcium oxalate, which is *insoluble* in acetic acid, but soluble in dilute nitric or hydrochloric acid and more slowly in sulphuric acid. These tests should be applied in watch-glasses, not on slides, as the acids corrode the stage of the microscope. They work well but slowly with the raphides of *Kniphofia*

¹ Large crystals of this kind, which end in a small flat surface, are known as *styloids*.

or the sphæro-clusters of *Æsculus* leaf-base. The latter are first disintegrated into their constituent crystals and finally dissolve completely in the stronger acids. The envelope of the bundle can be seen clearly in *Kniphofia* after solution of the raphides is complete.

32. Cystoliths and Calcium Carbonate. Cut a transverse section of the leaf blade of *Ficus elastica*; mount in water; examine the hypodermal region of the upper side. Draw under H.P. one of the exceptionally large cells which contain an oval ingrowth with a rough surface. This is a cystolith (see Fig. T 45). Place your section in a watch-glass with a little acetic acid and note, under L.P., the gradual solution of the incrustation of calcium carbonate together with the appearance of a distinct stalk and terminal bulb which is much smaller than the original cystolith.

Cell contents, other than those here mentioned, occur most conspicuously as reserve materials and are dealt with in Chapter XVIII.

33. The Nucleus and Nuclear Division. (a) Examine the prepared slides of root-tips carefully under the high power.

Distinguish and draw at least four different stages in vegetative nuclear division (**mitosis**) (*cp.* Fig. T 66).

(b) Examine the prepared slides of *Lilium* flower-buds. The young embryo-sacs of the ovules show various stages of **meiosis**.

The developing pollen-mother-cells of the young stamens also show similar stages. Draw at least four clear nuclear figures to show (1) the looped spireme of synizesis or synapsis; (2-3) the spindle and double chromosomes of later stages; (4) the tetrad of nuclei formed by meiosis (*cp.* Fig. T 67, which is only a diagram with a very heterogeneous collection of chromosome forms).

(c) Examine the prepared section of *Allium* root which is specially fixed and stained to show the chondriosomes, extra-nuclear bodies which when in the form of granules are called **mitochondria**. These may function as the initial bodies for various plastids and in other ways.

TISSUES

Drawing Directions

The student who has done the above exercises is already acquainted with several tissues, such as meristems Ex. 26,

epidermis Ex. 25, chlorenchyma Ex. 27, ground-tissue and conducting tissue Ex. 24, 26. He should meet these again and others during later exercises on the details of plant structure. It is important, however, that students should be able to recognise the various tissues as such and not merely as cells located in particular positions in stem, leaf, root, etc. It is important also, as a matter of training, that he should be able to record correctly the characteristics of

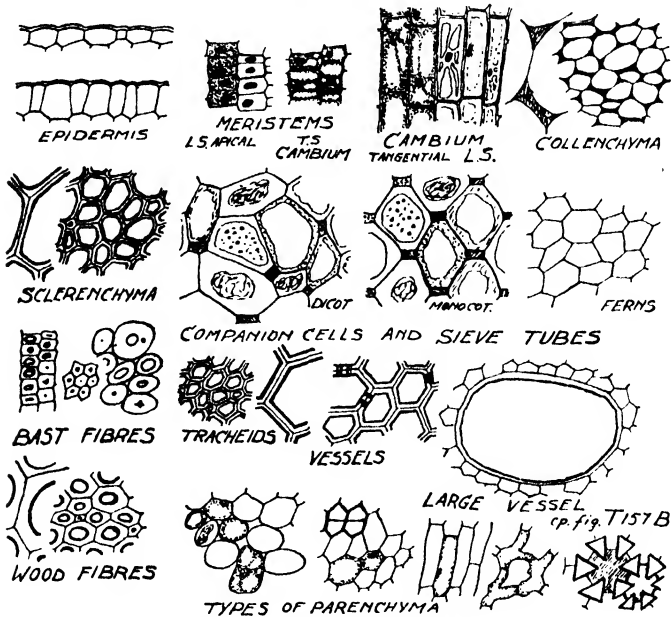


FIG. 7.—Tissue Drawing Methods for Students.

each tissue. The student should, therefore, note very carefully the following points: namely, (a) the relative size of the various cells, (b) the shapes of the cells as seen in transverse and in longitudinal section, and where possible in surface view, as in epidermis. These shapes are best considered as being made up of four or five or more sides; and the straightness or otherwise of the sides, together with their number and relative lengths, should be noted. This procedure enables a student who can write, but vows that

he cannot draw, to record with some degree of precision what he has seen. Actually, "if you can write you can draw," but you must know first of all whether you should be making an "a" or a "b"; in other words, the student who has observed properly and *knows what he has seen* can always put it down on paper.

As a guide to the usual characteristics of tissues (Fig. 7) we may take the following :—

Epidermis. In surface view, as on leaves, the walls are usually five or six in number and may be straight or wavy. There are frequently several long walls and several short walls. The various arrangements of lengths and curves and the angles at which these meet give various shapes which may be characteristic of the species or genus. On stems the epidermal cells are usually brick-shaped with four long and two short walls all more or less straight.

In section the epidermal cell has the outer wall curved, the lateral walls usually shorter and straight, and the inner wall may be single and straight or it may have two or three sections meeting at a rather wide angle. See Figs. T 64, T 70, T 149, T 151, T 255.

Advanced students should study the epidermis of *Aloe* leaf, *Mesembryanthemum crystallinum*, *Nerium oleander*, *Dianthus caryophyllus*, etc.

Meristems. Except in the lower plants, some of which have single apical cells of various shapes governing cell-multiplication, meristems are the tissues in which cell-division takes place. In the apical regions of stem and root these cells are almost cubical or flattened squares, so that they appear as squares or rectangles which may develop into pentagons or hexagons by mutual pressure of the cells. Apical meristem cells have characteristic dense contents with vacuoles absent or few and small. Meristematic tissue elsewhere in the plant usually shows a large proportion of dense protoplasmic contents, but these cells are frequently vacuolated and elongated to a considerable degree.

The characteristics of meristem cells are, therefore, the squarish or oblong shape, the rather dense granular contents with or without vacuoles and the presence of new thin walls, each stretching across an otherwise clearly defined individual cell. These cross-walls are frequently thinner than the other walls which surround the pairs of daughter-cells. A

clear sign of meristematic activity is to be seen in the orderly arrangement of cells in rows which may be longitudinal as in the apex of root and stem, or radial as in secondary growth. See Figs. T 68-71, T 149, T 151-152, T 154, T 394, T 398, T 402, T 405, T 408-410.

Advanced students should study meristems in the apex of *Hippuris stem*, root apex and lateral root primordia, grass nodes, etc.

Chlorenchyma. This tissue can always be distinguished in the fresh condition by its one essential, the presence of green plastids (chloroplasts). The shape of the cells varies very considerably, from globoid (Ex. 27) to an elongated oval as in palisade chlorenchyma, or to irregularly stellate as in spongy chlorenchyma. It occurs in leaves and towards the outside of many stems. See above, Ex. 27, for advanced work.

Laticiferous Tissues. In the fresh plant the presence of these tissues is indicated by the exudation of a milky fluid when the tissue is either broken across as in the dandelion or pricked with a pin as in spurges (*Euphorbia* spp.), etc. When this symptom is present the tissue may be found best in longitudinal sections, as a branching system of thick-walled tubes or as an irregular anastomosing system of relatively thin-walled cavities. In all types the latex shows up as dense, sometimes yellowish, contents. In transverse sections these tubes or cavities are cut across and the dense contents may have an opportunity to escape by reason of the elastic contractions of the walls. The thick walls of the rounded tubes can be seen and in fixed material the dense contents remain to indicate the transverse distribution of the laticiferous tissues, which are often associated with bast, but occur also elsewhere in the plant. See Figs. T 83-84.

Advanced students should study L.S. of *Euphorbia* stem, *Taraxacum*, *Euonymus* bark, *Lactuca* leaf, etc.

Sclereids. These are isolated cells with thick lignified walls. They vary from rounded shapes to elaborately branched structures which vary in the apparent shape according to the plane of section.

34. Separate from the soft tissues one of the many hard granules to be found in the flesh of a pear fruit. Squash this

granule firmly in a little water on a slide with the blade of a penknife. Mount the preparation with a cover-slip. Examine carefully with both L.P. and H.P. Draw one or two single sclereids, using H.P.

35. Cut a section across a rolled tea-leaf which has been soaked in hot water. Used tea-leaves suit admirably. Observe the variation in the apparent shapes of the sclereids. Tease out a portion of the leaf in water and examine this preparation. Draw a complete sclereid using the several views obtained for your three-dimensional reconstruction.

Hakea leaf, after simmering in dilute caustic potash, may be used instead of old tea-leaves. Compare also *Nymphaea stellata* petiole (Fig. T 85), *Camellia* leaf, *Sciadopitys* leaf, *Bignonia* hypodermis, *Plantago lanceolata* rhizome, etc.

Collenchyma. The essential feature of this tissue is the unequal cellulosic thickening of the walls which remain unligified. The nature of this thickening can be tested with chlor-zinc-iodine (blue = cellulosic) and with aniline sulphate (not yellow = unligified).

In drawing collenchyma the student should not allow the conspicuous irregular masses of the thickening to distract his attention from the fact that it is a tissue, a group of similar cells. He should first fill in lightly the outlines of the constituent cells in order to get them in the proper places and fitting together in the drawing as they do in the sections. Afterwards the angular blobs of thickening can be darkened according to the pattern shown, along mutual boundary walls of the cells or in the angles where three or four cell-walls meet. Very great care and absolute accuracy are required to obtain a reasonably good fit if the thickenings are drawn first, and the student should therefore follow the above instructions closely (see Fig. 7).

The advanced student should compare various petioles and stems of Umbelliferae, including *Astrantia* petiole, with *Helianthus* and *Lamium*.

Sclerenchyma. The essential features here are the lignification of the thick walls (yellow with aniline sulphate, etc.) and the characteristic elongated cells showing bluntly pointed ends in longitudinal section. The spindle ends to the otherwise nearly cylindrical cells show in transverse section as apparently smaller cells. Since there is much

more of the cylindrical lengths than of the ends, the bulk of this tissue appears in cross-section as thick-walled cells ; with polygonal outer sides to the walls, which should be drawn first to give the general position of the cells ; and with the inner line of the wall parallel to the sides of the outer polygons, but rounded instead of sharp at the angles. This inner line should be drawn last, giving three lines to each wall, a central light wall and a darker line indicating the inside of the two adjacent cells (see Fig. T 151 *p.f.*). Sclerenchyma varies very considerably and may be built of isodiametric cells, as in many fruits, or it may have a fibre-like structure, as in ferns.

Phloem. The essential elements of bast or phloem are the sieve-tubes ; the essential characteristic of which is the presence of sieve-plates. It is quite necessary to insist upon this point, as students and others sometimes consider that any group of cells with dense contents and unligified walls may be said to be phloem. In the angiosperms these sieve-plates may be seen in a few of the wider tubes in a transverse section ; but the sieve-tube tissue, like the latex tube, is more easily seen in longitudinal sections (*cp.* Figs. T 154, T 157). The transverse sieve-plates of angiosperms are more numerous and more conspicuous in L.S. ; and the sieve-plates on the lateral walls in some angiosperms (Fig. T 90) and in all gymnosperms and ferns can scarcely be found by the student except in longitudinal sections.

- In observing sieve-tubes for the purpose of recording their structure the student should note that in L.S. they are nearly cylindrical with oblique or bluntly pointed end walls ; in T.S. they are frequently five-sided with short and long sides in dicotyledons (Fig. T 88, T 149, T 151), frequently eight-sided with four short sides alternating with four longer sides in a very regular pattern in monocotyledons (Fig. T 156), and usually six- to eight-sided in the ferns (Figs. T 168, T 170).

Companion cells, showing as small rectangular cells with dense contents, accompany the sieve-tubes of higher plants, but are absent in ferns and gymnosperms.

Bast fibres may be drawn according to the directions for sclerenchyma (see above), making the outer line of the wall light and the inner line darker. Bast fibres vary considerably in shape, but they have usually acutely pointed ends and

often have thick lignified walls. In transverse section they may appear almost rectangular, rounded or polygonal. *The inner line of the wall is always rounded, not angular*, and the lumen or cavity may be reduced by the thickening to a small circle (punctiform) or to a narrow slit (linear).

Xylem. The essential elements of the wood or xylem are tracheids, which are cylindrical cells with oblique or straight end walls and thick lignified walls pitted or thickened in rings, spirals, ladders or networks. It is again necessary to insist upon this point, because students and others show a tendency to consider that angiosperms have vessels or tracheæ as their essential woody elements. Vessels are absent from most of the gymnosperms and all the ferns, but their presence in angiosperms does not as a rule mean the absence of tracheids, which remain to form the bulk of the wood in most conducting strands. In a few cases such as very young sunflower stems tracheids may be absent temporarily from the xylem.

Vessels and tracheids are very similar both in L.S. and T.S. They both have thick lignified walls showing as polygons in T.S., with the inner lines of the sides very strictly parallel to the outer lines and showing the same wide but sharp angles inside as outside. They are both thickened unequally and show in L.S. rings, spirals, ladders or networks of thicker parts of the wall. They both show pits of various kinds, including bordered pits with circular openings as in conifers or with crossed slit-like openings as in many angiosperms. They differ in two points: (a) the vessel is sometimes but *not always* larger in diameter than the tracheid; (b) the vessel has perforated cross-walls. The latter is the only essential difference between vessels and tracheids, and the perforation of the cross-walls may be complete giving one large hole, or scalariform giving a varying number of slits. In the latter case the absence of the middle lamella becomes the only criterion as to the nature of the cell, and the presence or absence of this fine layer is sometimes difficult to demonstrate.

In addition to vessels, other subsidiary elements sometimes occur in xylem. These may include thin-walled or thick-walled fibres which are unligified; and thick-walled spindle-shaped lignified wood-fibres which have the line of the lumen very definitely rounded and not parallel to the

polygonal outer sides in T.S., and in which the lumen may be reduced to a mere point.

Cambium and other Interstitial Meristems. These are generally composed of elongated tabular cells showing two short with two longer walls in T.S. and two short with two much longer walls in L.S. (*cp.* Figs. T 151–154; also Figs. T 405, T 408). Since active cell-division occurs with the new walls usually tangentially arranged, these tissues all show a characteristic arrangement of the cells in radial rows. The student should note that although in T.S. the cells are brick-shaped, their arrangement in T.S. is *not* that seen in a brick wall, where the bricks alternate in position as one passes at right angles to the long axis of the rectangles.

Endodermis. A modification of the brick-wall arrangement is shown by this special tissue which forms a hollow cylinder around the conducting system of the stem and root. The cells are more or less rectangular in T.S. and in L.S., the length being much greater along the axis of the organ than radially or tangentially. Position is sometimes the only characteristic of this tissue, but this layer of cells may contain starch grains (giving blue with iodine, Fig. T 151), or it may show suberised portions on the lateral as well as on the top and bottom walls (see Figs. T 149, T 151), or the suberisation may extend over the inner wall also and the wall become unequally thickened (see Fig. T 304). The suberin dots of the Casparian strip, as well as the more easily distinguished greater suberisation, can be seen clearly when stained with Soudan red or phloroglucin (see Appendix II).

Cork. Developed as the result of meristem activity cork cells show an unusually regular arrangement of the cells in radial rows. The cells are rectangular in shape, brown in colour and highly suberised (Figs. T 398, T 408–410).

36. (a) Cut a thin cross-section of a bottle cork; mount in alcohol, add dilute glycerol; and draw a sufficient number of connected cells to show the characteristic arrangement as well as the shape of the cells. (b) Cut thin transverse sections of elder (*Sambucus*) stem, and make careful H.P. drawings of the outer tissue to show the cork layer and the meristem layer from which the former arises.

Parenchyma. This thin-walled unligified tissue with granular cytoplasm and large vacuoles forms the ground-tissue of all organs. The thin wall should be represented in drawing by one thin line. The granular contents may be stippled. The shapes vary too much for specific drawing directions, but the student should note, as in other tissues, the number and straightness or otherwise of the sides of the polygons, and, in addition, the presence or absence of gaps (intercellular spaces) between the cells. Some of the variations are illustrated in Figs. T 72-81.

Tylosis. As a special type of irregular secondary parenchyma this occurs in the cavities of the tracheids and vessels of old wood.

37. Cut a longitudinal section of the timber supplied and examine it carefully. Some or all of the wood elements should show tyloses (see Fig. T 121).

Although a few exercises have been given for special tissues, the above details are given mainly to assist the student in the identification of tissues as specific tissues and to help him towards recording correctly the observations which he will be required to make in later exercises. There is much variation, and many exceptions or abnormalities will be encountered in any extensive course of histology. In any case the actual arrangement of the cells varies from plant to plant and **THE STUDENT SHOULD MAKE HIGH POWER DRAWINGS ACCURATELY CELL BY CELL FROM HIS OWN SECTION.**

CHAPTER VI

THE STEM

At this stage the first-year student should make the acquaintance of a complete plant and distinguish the main parts which make up the whole.

38. Examine the external features of the **groundsel** (*Senecio vulgaris*) or other plant supplied. Make a drawing to a stated scale of the entire plant showing (1) shoot with stem, leaves, nodes and internodes, buds and flowers; (2) root with root-branches or rootlets, dissimilar parts being absent in the root but present in the shoot. Annotate your drawing, labelling all the parts which you can recognise (*cp.* Figs. T 1, T 122, T 138).

39. The exercise corresponding to Ex. 38 for more advanced students is given in Chapter II. Each student should investigate a different species according to the outline given in that chapter.

40. Extending the elementary examination of the plant, as in Chapter II of the Textbook, a single example of a flower and a fruit may now be noted and the details recorded.

(A) Flower. Examine the flower of the **lesser celandine** (*Ranunculus ficaria*) and distinguish (a) three greenish sepals, (b) eight or more yellow petals each with a small honey gland at the base (Fig. T 708), (c) numerous stamens each with a filament and an anther containing pollen, (d) numerous small greenish carpels crowded together (Fig. T 455). Each carpel is made up of an ovary and a stigma for receiving pollen. The ovary contains one ovule which, after fertilisation, develops into a seed and the ovary becomes a fruit.

(B) Fruit. Examine and draw the fruit of **wallflower** (*Cheiranthus cheiri*). The fruit in this case has developed from two carpels joined together. Split the fruit lengthwise and draw a small portion showing several seeds attached by stalks to the wall of the fruit (Fig. T 767-768).

such rhizome to show at least two shoots or buds, together with the scale-leaves or leaf-scars which are present (see Figs. T 132-133).

(b) Root suckers are common around trees and bushes ; and may be seen in the open around poplars, willows, lilacs and wild roses or neglected garden roses. Examine a prepared specimen of one such sucker ; draw it carefully to show the presence of rootlets and the sucker together with the absence of scale-leaves or leaf-scars.

47. Rhizomes. (a) Make a diagram of the prepared specimen of the monopodial rhizome of the bracken fern (*Pteris aquilina*). Note the single leaf and the horizontal growing-apex.

(b) Make a careful drawing of the apical portion of the sympodial rhizome of *Iris*, or *Convallaria* (Lily of the Valley) or *Polygonatum* (Solomon's Seal) ; showing the leaf-scars, the rootlets (adventitious), also particularly the details of the scars of the old flowering shoots and the manner in which the terminal bud turns up (see Fig. T 134-136).

48. Erect Rhizomes and Root-stocks. Make diagrammatic sketches showing (1) the position relative to ground level, (2) the leaf-scars, (3) the rootlets and (4) the pull-roots in the following : *Taraxacum officinale*, *Oxalis acetosella*, *Ranunculus bulbosus* (see Figs. T 1 and T 137-138).

49. Soboles. Make a careful drawing of at least three nodes and two internodes of the thin rhizome or sobole of one of the following : couch-grass (*Agropyron repens*), marram grass (*Ammophila* or *Psamma arenaria*), sand sedge (*Carex arenaria*). Your drawing should show clearly (1) the scale-leaves, (2) the rootlets, (3) one current leafy shoot and (4) the terminal bud (see Fig. T 139).

50. Tubers. (a) Examine carefully and draw the tuber of the potato plant (*Solanum tuberosum*) ; note the stalk-scar at one end and the gradually increasing frequency of " eyes " as you pass from that to the other end, where there is one terminal eye-bud which has no corresponding leaf-scar and which is surrounded by several other buds or eyes. The apical eye consists of a single bud. Examine and draw on a large scale one lateral eye to show : (1) the leaf-scar with or without the ragged remains of a scale-leaf, (2) the central main bud of the eye, (3) the two lateral buds in the axil of the same scale-leaf, (4) any other accessory axillary buds

which may be present ; there may be as many as nine buds in one " eye " (see Fig. T 140).

(b) Examine and draw the tuber of the Jerusalem artichoke (*Helianthus tuberosus*) ; note that here the scale-leaves persist. They are often large and fleshy, covering the relatively large axillary buds. Dissect off several scale-leaves ; make a drawing of one axillary bud and its associated scale-leaf which you have already dissected away (see Fig. T 141).

(c) Examine and draw the underground storage stem of the false oat-grass (*Arrhenatherum elatius*) to show (1) the swollen tuberous internodes, (2) the leaf-scar at the base of one such internode, (3) the axillary bud if present in the specimen.

51. Corms. (a) Examine a *Crocus* corm carefully ; note that it is covered with brown scale-leaves which are mainly attached towards the base and towards the top of the corm. The line of insertion of each leaf is quite long, nearly encircling the corm. Dissect off the brown scale-leaves and look for small axillary buds. Note the main apical bud. With a sharp knife cut downwards through one of the larger axillary buds, cutting part of the corm away. Note the solid stem of the corm and the conducting strands passing to the buds. Dissect the apical bud carefully, noting the outer fleshy colourless leaves, the small pale yellow or greenish foliage leaves within, and the coloured flower-bud in the centre (Figs. T 142-143). Make annotated or labelled drawings of all your dissections.

(b) Examine the corm of *Montbretia* (*Tritonia*) ; note that this is very similar to the above in general construction, but shows no young flowers in its apical bud. This corm also shows a very definite basal stalk and may have a secondary corm on a slender stalk attached near the top of the parent corm. In the crocus the secondary corms are sessile developments of the axillary buds which occur around the top of the parent corm, but in *Tritonia* the axillary bud develops into a long, thin, tough branch with one or two scale-leaves, which branch swells up at the end to become a daughter-corm. Make an annotated diagram to show the relations of the parts, labelling as in the crocus.

(c) Pharmaceutical students, in particular, should also examine the corm of *Colchicum autumnale* where the struc-

ture is similar to the crocus bud, but the flower bud is lateral instead of apical.

52. Bulbs. (a) Examine carefully the bulb of the tulip or daffodil. Cut it in half along the centre. Make an annotated diagram to show (1) the flattened and relatively small solid stem, (2) the brown outer scale-leaves or tunic, (3) the fleshy inner scale-leaves or leaf-bases which encircle the bulb-centre and have broad lines of insertion, (4) the pale green foliage leaves which are less wide and have smaller lines of insertion, (5) the central flower-bud (cp. Figs. T 144–147).

(b) Compare with the tulip the bulb of the onion (*Allium cepa*), snowdrop (*Galanthus nivalis*), the bluebell (*Scilla non-scripta*) or the squill (*Scilla verna*). The structure of each of these is similar to that of the tulip, but the number of scales varies and the foliage leaves are narrower.

(c) From your studies of tubers, corms and bulbs, prepare a set of three diagrams to show the relative size of stems, scale-leaves, axillary buds, and apical buds in these three types of storage organs.

STEM STRUCTURE OF A DICOTYLEDON

53. Cut a number of transverse sections of the stem of the sunflower. Stain one in aniline sulphate and mount it in dilute glycerine. Mount one in chlor-zinc-iodine. Make an annotated L.P. line diagram (Fig. T 150) to show :

I. Epidermis. A single peripheral layer of cells. **II. Cortex.** Consisting of (a) collenchyma, (b) parenchyma with air-spaces, (c) endodermis—a layer of cells containing starch grains (staining blue in chlor-zinc-iodine). **III. Stele.** Consisting of (a) vascular bundles in one peripheral ring embedded in (b) parenchymatous ground tissue which forms a large pith and numerous medullary rays separating the vascular bundles from each other. Note and draw carefully UNDER HIGH POWER (Fig. T 151) the following :—

1. **Epidermal cells** with thin layer of cuticle on the outside.
2. **Collenchyma**, the walls of which consist of cellulose and are thickened at the corners.
3. **Parenchyma.** Thin-walled assimilating tissue of the cortex; note decolorised chloroplasts, also intercellular spaces.
4. **Endodermis.** Note starch grains inside and lateral walls thickened.

5. **Pericyclic fibres** (sclerenchyma) with thick lignified walls and small rounded cell-cavities.

6. **Phloem**, consisting of (a) sieve-tubes and companion cells, (b) phloem parenchyma.

7. **Cambium**, between the xylem and phloem. The thin-walled cambium produces externally new phloem and internally new xylem elements.

8. **Xylem**. Note (a) large lignified vessels of the metaxylem, and (b) smaller vessels of the protoxylem which abut on the pith (compare position of the protoxylem in a root), (c) xylem fibres (polygonal cavities in T.S.) with thick lignified walls and without protoplasm, (d) xylem parenchyma with cellulose walls and protoplasm.

9. **Pith**, with parenchymatous cells progressively larger towards the centre of the stem and few air-spaces amongst the cells. N.B.—Refer to Fig. 7 and the corresponding text before beginning your H.P. drawing.

54. Examine the stem of the **deadnettle** (*Lamium*) and make an annotated L.P. line diagram together with a H.P. drawing according to the scheme given above in Ex. 53 (see Figs. T 148–149).

STEM STRUCTURE OF A MONOCOTYLEDON

55. (A) **Transverse Section**. Examine the prepared slide supplied of T.S. stem of *Zea mais* (Indian Corn). Make a L.P. line diagram to scale showing—(1) Epidermis, (2) Narrow Cortex, (3) Large Stele with numerous scattered vascular bundles. Compare the arrangements of the bundles in a Dicotyledon. Under H.P. draw carefully one of the larger vascular bundles near the centre and observe—(a) the V-shaped xylem consisting of two large metaxylem vessels (these are pitted) and two smaller protoxylem vessels (one is annular and the other annular or spiral). The vessels are embedded in xylem parenchyma. On one side of the protoxylem there is usually a prominent space which in the living plant contains water. (b) The phloem (lying between the two large metaxylem vessels) consisting of relatively large, clear sieve-tubes, each one of which is accompanied by a small companion-cell with abundant protoplasmic contents. (c) The bundle sheath surrounding the xylem and phloem is a sheath of sclerenchyma partly interrupted by thin-walled cells opposite the phloem (Figs. T 155–156). Explain the significance of the fact that in both Sunflower

and Maize the sclerenchyma is found chiefly round the periphery of the section. Note carefully the absence of cambium between xylem and phloem in the monocotyledon.

(B) Longitudinal Section. N.B.—Longitudinal sections are always more difficult to interpret than transverse sections. All transverse sections of a given vascular bundle would be alike, but longitudinal sections through the same bundle will vary in appearance according to the plane of cutting.

Identify the structures seen in L.S. and determine accurately the plane in which the bundle has been cut (Figs. T 157, A and B, and Fig. T 115). Make H.P. drawings to show especially (a) annular or spiral vessels of the protoxylem, (b) a large pitted metaxylem vessel (probably in a different section), (c) sieve-tubes of the phloem with transverse sieve-plates (staining deep red with eosin), (d) narrow companion cells, (e) thick-walled prosenchymatous fibres of the sheath of sclerenchyma.

56. Cut several transverse sections of couch-grass rhizome (sobole). Stain one in aniline sulphate and another in iodine solution. Make a line diagram to show: (1) the distribution of vascular bundles, (2) the distribution of sclerenchyma (see Fig. T 158).

FURTHER EXERCISES IN STEM STRUCTURE

57. **Structure of Cucurbita Stem.** (a) Cut several sections of Cucurbita stem. Mount one in chlor-zinc-iodine. Stain another in aniline sulphate and mount in glycerine. Observe under L.P.—(1) Epidermis; (2) Cortex, which is widest opposite the ridges of the stem; opposite the furrows it is only a few cells in width. Opposite the ridges it has an outer zone of collenchyma and an inner zone of thin-walled parenchymatous cells. The innermost layer of the cortex forms an ill-defined starch sheath, which is the endodermis. (3) Stele: the stele is defined by a band of sclerotic cells, the outer pericycle; the inner part of the pericycle is thin-walled parenchyma. The vascular bundles form two series, the inner bundles being larger. Compare this with the single ring in sunflower. Examine one of the large bundles under H.P. and note that it is bi-collateral, *i.e.* there is a strand of phloem on the inside as well as on the outside of

the xylem. The protoxylem is endarch, *i.e.* at the inner margin of the xylem. The xylem has unusually large vessels. Note that cambium occurs between the outer phloem and the xylem. Traces of cambium may also be found between the xylem and the inner phloem; this inner cambium is sometimes very well-developed in old stems. Examine the phloem carefully and note (1) Sieve-tubes—elements with large lumens; transverse sieve-plates may be seen; these have a punctate appearance due to perforation by the sieve-pores: (2) Companion cells are small cells attached to the sides of the sieve-tubes; they are sister cells of the sieve-tubes and still appear as though recently cut off from them by a longitudinal wall: (3) Phloem-parenchyma, which forms the rest of the phloem. In sections treated with chlor-zinc-iodine all the walls of the phloem turn blue, but the sieve-plates appear yellow or brown owing to the callus mass round them (Figs. T 153 and T 88). Make a low power line diagram of the section and careful high power drawings of representative cells.

(b) Cut radial longitudinal sections through a vascular bundle; mount in iodine solution. Note and draw transverse sieve-plates and the callus mass which invests them. Draw also at least three kinds of thickening as shown in L.S. of vessels (Fig. T 154).

58. Pocket-lens Observations. Even the first-year student will acquire an interesting knowledge of the larger features of plant anatomy, if he takes a sharp knife or a pocket-razor (utility knife) and his pocket-lens with him during country or garden walks, and observes as follows: Cut a thin slice of stem, leaf-stalk or other part of the fresh plant; impale this upon a pin or hold it with a pair of spring tweezers (cover-glass tweezers or artery forceps are most convenient); hold the thick section or slice up to the light and examine it carefully with a pocket-lens.

In this way he will find that—

(a) Sunflower seedling hypocotyl has a single ring of bundles:

(b) Pea seedling stem has four bundles in a square and shows the passing outwards of leaf-trace bundles; this can also be traced in longitudinal slices:

(c) Young stems of shrubby species of *Veronica* show very clear leaf-traces; the gap left can be seen easily and the rapid filling up of this gap can be followed by taking a

series of four or five slices; longitudinal slices taken radially also show this clearly:

(d) A similar series of slices, taken through two successive nodes of *Lamium* stem, shows the four corner *cauline* bundles passing practically undisturbed through the nodes, while the side bundles pass out as leaf-traces; *cauline* bundles do not leave the stem and occur in many other cases:

(e) *Buddleia globosa* stem shows a square stem with an anatomy like that of *Lamium* (Fig. T 148), but with the outer part of the stem ringed round with a felt of large stellate hairs, easily seen with a pocket-lens:

(f) Wallflower (*Cheiranthus*) stem shows a pentagon of conducting tissue:

(g) London Pride (*Saxifraga umbrosa*) shows large easily seen cells in the cortex and in the stellate pith; the latter being surrounded by an opaque white band of sclerenchyma; the angle-bays of the star containing dark masses of wood and the endodermis showing clearly as a thin pink line round the central mass:

(h) Groundsel (*Senecio vulgaris*) stem shows a peripheral ring of bundles like sunflower, but with very large cells in the pith, easily distinguished as cells under the lens:

(i) The tuberous roots of *Ranunculus ficaria* tubercles have an opaque central strand, showing that they are roots not stems:

(k) Scattered bundles are to be found in unexpected places, and it is really only *scattered bundles with no cambium between xylem and phloem* which can be taken as indicating a monocotyledon. Thus he will find many scattered bundles in the leaf-stalks of rhubarb. *Rumex* sp. (docks), butterbur (*Petasites*), in both leaf stalk and stem of the giant fennel and some other Umbelliferae, although hemlock (*Conium*) leaf-stalk has only one ring of big bundles. He will find the typical scattered arrangement also in *Asparagus* stem, *Iris* rhizome especially near the apex, and so on; one ring only in aconite rhizome, but one ring of bundles with xylem dark and phloem opaque white or grey as usual in the rhizomes of plantain and dock (*Rumex*) with leaf-traces in the cortex. The plantain (*Plantago lanceolata*) also has scattered dark masses of sclereids in the pith which do not look at all like conducting strands even under the lens. The dock rhizome has many scattered bundles in the cortex and the slice is very like a section of couch-grass on a larger scale (Fig.

T 158), but the bundles under the microscope show cambium in *Rumex* and no cambium in *Triticum repens*. He will also find hosts of other interesting phenomena, as shown in "Pocket-lens Plant Lore."

59. Examine a prepared transverse section of the stem of pepper (*Piper*) or of the rhizome of dock (*Rumex*). Draw a diagram to show the two rings of bundles in the pepper or the scattered arrangement in the dock. Make a H.P. drawing of a portion of one of the bundles to show the intrafascicular cambium in either example.

60. Cut transverse sections of a solanaceous stem, such as potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), bittersweet (*Solanum dulcamara*), or of any other genus of the family (see Textbook, pp. 603-604). Make accurate H.P. drawings to show the internal phloem and a L.P. line diagram to show the distribution of that tissue.

61. Examine as in 60 the bicollateral bundles of gentian, centaury, chiretta, *Chlora* or myrtle (see Textbook, pp. 599 and 593).

62. Examine carefully a prepared transverse section of a young stem of *Pinus*. Make a line diagram to show the similarity of this to a dicotyledonous stem.

63. Examine a prepared transverse section of a portion of an *Aspidium* rhizome. Note the absence of cambium and the arrangement of protoxylem, xylem and phloem. Look for internal glands. Make an annotated line diagram of the section (*cp.* Figs. T 166-168).

64. Make annotated line diagrams, combining the appearances in T.S. and L.S., to show the arrangement of the mechanical supporting tissues of the stems of *Lamium*, *Cyperus*, *Juncus*, *Allium* (scape), *Molinia*.

65. On the basis of your observations prepare a table to show the real differences between the stems of dicotyledons and monocotyledons, the position of the protoxylem, xylem and phloem in relation to one another in the stems, and the external characteristics of the stem as a main axis, or as a branch axis arising in the axil of a leaf, giving rise to lateral organs which are essentially different from the stem, *e.g.* leaves.

CHAPTER VII

THE LEAF

General Foliage-Leaf Structure

66. MAKE careful drawings of the leaves of the following plants: (1) Dicotyledons—Rhododendron, Ivy, Buttercup, Horse-chestnut. (2) Monocotyledons—Grass and Daffodil.

Note: (a) Whether leaf is simple or compound; (b) whether leaf is petiolate or sessile; (c) margin of lamina—entire, toothed or lobed; (d) nature of leaf-base; in grass note the ligule; (e) primary venation—pinnate, palmate or parallel.

Note the secondary reticulate venation of the leaf of the Dicotyledon. Draw the main veins of each leaf and make a large-scale drawing of part of each leaf to show details of veining.

67. Examine and make an annotated drawing of a foliage leaf of the rose, as an example of a complete leaf, showing leaf-base, stipules, petiole, and lamina. The lamina is completely divided, so that this is a compound leaf, with the main midrib as a rachis to which are attached a varying but usually unequal number of leaflets. Note also the trichome or hair-like prickles if they be present on the rachis (Fig. T 173). Observe carefully and thoughtfully that the lamina is **broad** and **flat** and **thin** and **green**; these are the characteristics of a foliage leaf-blade, which are essential for the proper carrying out of its functions.

68. Strip off a small portion of the **epidermis** of daffodil foliage leaf and mount in water with the outer surface uppermost. Under lower power note: (a) ordinary **epidermal cells**; (b) **stomata** arranged in longitudinal rows. Under H.P. draw (a) a few epidermal cells, noting the cell-wall, the granular protoplasm and a round or pointed nucleus containing one or more nucleoli; (b) a single stoma, showing also the two guard-cells which contain chloroplasts. The guard-cells enclose the slit-like stoma or pore.

69. Mount a leaf of *Elodea* in water. Under H.P. note the numerous **chloroplasts** in each cell. These may be found embedded in actively circulating protoplasm in each cell. Draw a single cell showing the contents.

70. Examine a leaf of **Box** (*Buxus sempervirens*), which has been boiled for ten to fifteen minutes in potash solution. Separate it into three layers, (a) upper epidermis, (b) mesophyll, (c) lower epidermis. Mount each layer separately in dilute glycerine. Draw and compare the upper and lower epidermis as to presence or absence of stomata. Draw the arrangement of veins and vein-endings as seen in the mesophyll under L.P.

71. Examine a prepared section of the leaf of **Rue** (*Ruta*). Note under L.P. and H.P. : (a) upper epidermis—a single layer of cells with cellulose walls, the outer wall covered and thickened with cuticle : (b) palisade parenchyma—one or two layers of columnar cells with cellulose walls : (c) spongy parenchyma—loosely packed irregular cells with intercellular spaces : (d) numerous small vascular bundles (veins) embedded in the spongy mesophyll ; these have xylem towards the upper epidermis and phloem towards the lower : (e) lower epidermis—similar in structure to the upper epidermis, but with smaller cells. In some sections stomata may be seen in transverse section. Note the two guard-cells and the pore or stoma leading to a large intercellular space. Make a L.P. line diagram of the whole section, also a H.P. drawing of a part including a vascular bundle (Fig. T 255).

72. Advanced and pharmaceutical students should know the use of the terms given in Appendix II of the Textbook, particularly in relation to the technical description of leaves. Practice descriptions of at least eight different leaves should be written out and checked. These should include, as far as possible, the arrangement, insertion, composition, as well as shape, margin, venation, apex, base, surface, texture, glands and hairs, colour, odour and taste.

73. **Hairs.** These are frequently of value in the identification of fragmentary specimens of leaves or other parts of the plant. The student should learn to distinguish practically the following types :—

Clothing Hairs. (a) Simple, unicellular, bicellular or multicellular.

(b) Branched, stellate or candelabra.

(c) Capitata, stellate or scale-like.

Glandular Hairs—with oil or other secretory products—

(d) Sessile or with a *short* unicellular stalk, and the glandular head composed of one cell to eight cells, commonly four.

(e) Long-stalked with one or usually more cells in the stalk, and several but usually one cell in the glandular head.

(f) Sessile, bladder-like, larger than either (a) or (b) and with thinner walls.

Cut very thin transverse sections of the rosemary (*Rosmarinus*) leaf; mount them in a drop of alcohol to get rid of air, add water and a cover-slip.

Identify the three kinds of hairs (b, d, f) which are present (see Fig. T 270).

74. Advanced students will find clothing hairs of type (a) very common; of type (b) on mullein (*Verbascum thapsus*), horehound (*Marrubium vulgare*), young ivy (*Hedera helix*), etc.; of type (c) on the leafy crown of pineapple (*Ananas sativa*), as well as *Tillandsia* and other Bromeliaceae, on leaves of alpenrose (*Rhododendron ferrugineum*), etc.; and glandular hairs of type (d) on leaves of *Scrophularia aquatica*, *Gratiola officinalis*, *Prunella vulgaris* and many others; of type (e) on leaves of *Verbena*, *Teucrium scorodonia*, *Lycopus*, *Digitalis*, *Hyoscyamus* and *Atropa Belladonna*, etc.; of type (f) on leaves of *Mentha* spp., *Thymus*, *Origanum*, *Stachys*, *Teucrium*, and many other aromatic leaves which have *no* clear oil dots showing when held up to the light.

75. **Stomatal Development.** The stages in the formation of stomatal guard-cells can be traced near the leaf-base of many monocotyledons, e.g. Hyacinth and *Tradescantia* and near the base of young *Sedum* leaves (cp. Figs. T 264-265; also Chap. XL.).

76. **Mechanical Tissues.** The foliage leaf as a broad flat expansion is subject to shearing strains. Consider the reticulate venation of a dicotyledon-leaf from this point of view. Examine by means of vertical transverse and longitudinal sections the arrangement of mechanical tissue in the leaves of *Zea mais*, *Iris*, *Eriophorum*, and *Phormium*, or such other monocotyledon leaves as may be available. Make annotated diagrams to show the girders and their connections if any.

77. Make line diagrams of the petioles of at least eight different kinds of leaves, as seen with a pocket-lens when cut across with a sharp knife. Note the general U- or V-shape of these cross-sections together with the general arrangement of the conducting strands. The latter varies more than the general shape does. The petiolar furrow may drain water from the leaf-blade towards the centre part of the root, and it also acts mechanically in leaf support; as may be seen by comparing the flexibility of a strip of paper 20 cm. by 1 cm. with the relative rigidity of the same strip after it has been folded lengthwise along the middle to form a long V.

78. **Hydathodes.** Examine with a pocket-lens (or the low

power of the microscope) the leaf-margins of lady's mantle (*Alchemilla*), hare-bell (*Campanula rotundifolia*), French or runner-bean (*Phaseolus*), or London Pride (*Saxifraga umbrosa*), paying particular attention to marginal vein-ends. Note and draw the glands which occur. These are *hydathodes*; see also Ex. 495.

79. Prepare vertical longitudinal sections of one or other of the above glands and make a careful drawing of the details as seen under the microscope (cp. Fig. T 897).

80. **Pocket-lens Observations.** Using a pocket-lens for your observations, make line diagrams of thin slices of the three main types of internal leaf-symmetry, namely (1) **dorsi-ventral** as shown by *Buxus*, *Hedera* (ivy), *Ilex* (holly), or almost any other ordinary leaf; (2) **isobilateral** as shown by *Iris*, *Eucalyptus*, *Dianthus caryophyllus* (carnation), *Eriophorum* or another leaf which is flat and vertical or nearly so; (3) **centric** as shown by *Juncus* (rush), onion, *Sedum* spp. (round-leaved stone-crops), *Mesembryanthemum* spp., *Hakea*, or other leaf which is more or less cylindrical and vertical.

81. Examine and make line diagrams of the cross-sectional appearances presented by various grasses under a pocket-lens, when the stem, together with its enveloping leaves, is cut across with a sharp knife just below the top of the first leaf-sheath. The following key gives an approximate idea of these cross-sections for common grasses:—

A. Leaves folded in V-section, giving flattened shoot.

- | | |
|--|---|
| (a) Close V—with ridges on both leaf-surfaces | <i>Aira cæspitosa</i> . |
| —with 9–12 adaxial sharp ridges and a flat, red sheath | <i>Lolium perenne</i> . |
| —with 4–6 adaxial sharp ridges | <i>Festuca ovina</i> . |
| —with adaxial flattened ridges | <i>Cynosurus cristatus</i> . |
| —with thick obtuse conspicuous ligule | <i>Nardus stricta</i> . |
| (b) Open V—smooth, keeled below with subsidiary furrow over midrib | <i>Poa trivialis</i> (ligule acute),
<i>P. pratensis</i> (ligule short). |
| —smooth, keeled below, with no subsidiary furrow | <i>Dactylis glomerata</i> . |
| —with flattened ridges | <i>Festuca rubra</i> . |

B. Leaves coiled in section (with adaxial ridges) giving rounded shoot.

(c) Half-coiled with V at midrib

- | | |
|---|--|
| with 4-6 wide ridges, hairy,
sheath round, white with
red veins | <i>Holcus lanatus.</i> |
| with 9-12 rounded ridges,
hairless, sheath round and
red | <i>Lolium italicum.</i> |
| similar, conspicuous keel
below midrib of blade,
sheath keeled | <i>Arrhenatherum
elatum =
Avena elatior.</i> |
| similar, blade keel not con-
spicuous, sheath not keeled | <i>Avena flavescens.</i> |

(d) Coiled round, only a little more than once—

- | | |
|--|--|
| with ridges on both leaf-sur-
faces; ligule long, hairy on
back, smooth-edged on back
ligule short, sheath brownish | <i>Agrostis alba
Alopecurus
pratensis.</i> |
| with adaxial ridges low and
flattish, ligule long, lacinate-
edged, glabrous. | <i>Phleum
pratense.</i> |
| thick in section rough-edged,
ligule short | <i>Festuca
pratensis.</i> |

(e) Coiled almost twice—

- | | |
|--|-----------------------------------|
| without obvious midrib, ligule
large with two tufts of hairs
at base | <i>Anthoxanthum
odoratum.</i> |
| with distinct midrib, ligule
very short | <i>Triticum
repens.</i> |

See "Pocket-lens Plant Lore," No. 93-96.

82. Examine prepared specimens showing the **change of leaf-form** in seedlings from the cotyledons to the mature foliage leaf, *e.g.* in broad bean, pea, lupin, etc. Make line diagrams of the various leaf-forms.

83. Examine and draw a prepared specimen of *Acacia melanoxylon* or other similar species, showing the transition from a petiolate compound leaf to a simple **phyllode**.

84. On the basis of your own observations write out a table of the various leaf-forms, with examples and notes on the essential and distinctive features of each kind of leaf. Add to this table your own method of deciding whether a given part of the shoot is a stem or a leaf, however modified.

CHAPTER VIII

THE ROOT

85. MANY roots have already been seen by the student in previous exercises ; these should be revised at this point, using the drawings already made.

86. **Root Hairs.** Examine and draw seedlings of Barley and Flax which have been grown in a damp atmosphere. Note the delicate root-hairs. Make large-scale drawings to show the distribution of the root-hairs (Figs. T 279-280).

87. **Root Cap.** Examine and draw the loose thimble-like root-cap of duckweed (*Lemna*). Note that it is attached only at the tip. Since these root-caps are not worn away by friction with the soil, they remain large as seen and are only vestigial parts of the roots, produced because the land ancestors of the duckweed had them (Fig. T. 278).

88. Make a diagram on a *reduced* scale of the very large root-cap of the prepared specimen of the root of screw-pine (*Pandanus*).

89. Note and make line diagrams of the examples supplied of **tap-root and fibrous root systems** (Figs. T 281-284).

90. Draw carefully the annulated root of ipecacuanha (*Uragoga*). Cut a thin slice and apply iodine solution. Note the wide starchy cortex and the small central dense circle of wood (Fig. T 286).

91. Examine the **tuberous adventitious roots** from the underground base of the lesser celandine. Compare these very carefully with your drawings of the bulbils (tubercles). Note that some of these roots have developed adventitious buds near the adaxial end and that others have no buds (early spring condition). These adventitious buds are not associated with a leaf-scar as are the similar but axillary buds of the tubercles. Make a large-scale drawing of one root with a bud.

92. Place portions of the leaves of *Bryophyllum*, *Begonia*, or *Cardamine amara* (the bitter cress of gardens) upon moist soil and cover with a low bell-jar, or use the simple germinator (Fig. 5). Note and draw the development of **adventitious roots and buds**.

93. Examine and draw the prepared specimen of an orchid root, showing young and old tubers (Figs. T 287-288).

94. Root of Monocotyledon. Examine a prepared slide of T.S. root of *Funkia* or other Monocotyledon. Make a line diagram of the whole section and an accurate high power drawing of a part of the section showing cell-detail.

Note: (1) Piliferous Layer: (2) Exodermis: (3) Cortex with a very distinct Endodermis (Fig. 304): (4) Stele with numerous xylem groups alternating with phloem groups, the protoxylem (small-celled) being external to the ordinary xylem (*metaxylem*); the cells of the stele immediately inside the endodermis form the Pericycle: (5) The central Pith (cp. Fig. 307).

95. Root of Dicotyledon. Cut transverse sections of the root of Buttercup (*Ranunculus repens*). Mount one section in dilute glycerine; a second in chlor-zinc-iodine; and stain a third with aniline sulphate and then mount it in dilute glycerine. Under low power, recognise the same succession of tissues as in a Monocotyledon root, noting the following differences: (1) the small number (three to five) of alternating xylem and phloem groups: (2) no pith is present; the large vessels of the metaxylem occupy the centre of the section, the protoxylem being again exarch or external to the rest of the wood.

Make a line diagram indicating the relative position and extent of each tissue present; and make a high power drawing of the stele (cp. Fig. 302).

96. Examine carefully a main root of the broad bean or garden pea, which has produced a number of **lateral rootlets**. Observe that the rootlets occur in from three to five (or in the bean rarely seven) rows. Cut a number of thin transverse sections of the main root near the lowest of the lateral rootlets. Mount several sections in water or dilute glycerine and look for the initial stages in rootlet formation (see Fig. T 306). Make a line diagram to show the relation of these root-initials to the protoxylem groups of the wood.

97. Cut a series of transverse sections of the stem of ivy (*Hedera*) or *Ficus repens*, in the region of the **aerial climbing roots**. Examine several sections and make a line diagram to show the internal (*endogenous*) origin of these rootlets. Sketch a small piece of stem with rootlets to show the distribution of the rootlets and leaves (Fig. T 294).

98. Examine and draw a prepared section of the **aerial root of an orchid**. Note particularly the velamen, and the passage-cells of exodermis and endodermis (see Fig. T 296).

99. Examine and make a line diagram of a prepared section of the **haustorial roots** of *Cuscuta*, or yellow-rattle (*Rhinanthus*) or *Bartsia*, within the host tissues; showing clearly in your drawing the relation of the tissues of the parasite to those of the host (*cp.* Figs. T 297-301).

100. Draw carefully several small branches of the prepared specimen of beech (*Fagus*) rootlets enveloped in **exotrophic mycorrhiza** (Fig. T 291). These may be found by removing the dead leaves from depressions around beech trees.

101. Examine and make a careful H.P. drawing of the outer cortical zone of a prepared section of *Fagus* rootlet to show the position of the **mycorrhizal fungus** with relation to the root tissues.

102. **Endotrophic Mycorrhiza.** Make a careful H.P. drawing of a prepared section to show the mycorrhizal fungal hyphæ in the root-cells either of *Calluna* or other ericaceous plant or of *Orchis mascula* or other underground absorbing orchid root.

103. Study the **transition in the vascular system** as it passes from stem to root in a young seedling of sunflower (*Helianthus*), by means of a series of comparatively thick sections taken one after another. A series near the bases of the cotyledons shows how the four bundles of each fuse, the central pairs of each coming together, and lower down in the stem the adjacent lateral bundles of the two cotyledons approximate; then all four groups pass into the ring of bundles in the stem. A similar series near the base of the hypocotyl will show the lateral and outward displacement of the protoxylem with fusion of the metaxylem groups to give a central xylem core, star-points of exarch protoxylem and alternating bundles of phloem (*cp.* Fig. T 309).

104. On the basis of your own observations write out a table showing the real differences between the roots of dicotyledons and monocotyledons, the position of the protoxylem, xylem and phloem in relation to one another in the root, and the external characteristics of the root as a main axis giving rise only to lateral organs which are essentially the same as itself. Add to this table notes on the various forms of roots and also your own method of deciding whether a given part of the plant is a root or stem or leaf, however modified.

CHAPTER IX

THE TRANSPIRATION CURRENT AND ASSOCIATED PHENOMENA

THE Transpiration Current as expounded in the Textbook involves many stages and phenomena from gaseous diffusion of water vapour through the stomata of the leaves to absorption of water by the root-hairs ; associated with these are the selective absorption of inorganic materials in solution, the general phenomena of inorganic nutrition, turgor of cells, turgescence rigidity of plant organs and many other points in the physiology of the plant in relation to water. Although these may be discussed logically from the root-absorption upwards to the leaf, another possible method of approach is the one adopted here, beginning with the demonstration of actual transpiration or loss of water vapour by the leaf and proceeding downwards towards earlier phenomena and fundamental causes of the effects observed.

Loss of Water Vapour

105. Place a leafy plant growing in a pot in an aluminium shell and cover with sheet rubber, leaving only the leafy shoot outside the waterproof chamber thus formed. Cover the whole with a bell-jar, leave overnight and note the condensation of water on the inner side of the bell-jar. A control bell-jar without a plant will show little or no condensation of water at any time. The loss of water, as water vapour, by the leafy shoot is therefore indicated.

106. Water loss through Stomata. Choose a leaf in which the stomata are confined to the under surface, *e.g.* lilac, cherry-laurel or other laurels ; affix closely to both under and upper surfaces pieces of blue cobalt paper. Leaf-clasps specially designed to give a close application of the paper to the leaf surfaces should be used.¹ It will then be found

¹ Those designed by the writer and figured are supplied by Messrs. Robinson, Nelson & Co., Manchester.

that the paper on the under surface turns pink, *i.e.* becomes moist, within half an hour, while the other remains blue.

107. Loss in Weight. Select a pair of similar large leaves growing on a bush, *e.g.* from the larger laurels, such as cherry-laurel, *Ficus elastica*, or other type with stomata only on one surface; cut them off and seal the cut ends by dipping them in melted hard paraffin; attach threads for suspension to the stalks. Weigh each leaf carefully. Hang the two leaves up in a

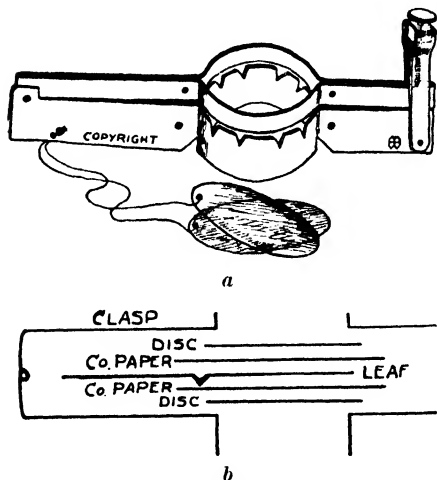


FIG. 8.—Small's Leaf-clasp. Type A.

(a) General view. (b) Diagram of Experiment 106.

dry dark place for two hours; weigh again and note loss of weight. Now smear the under surface of one and the upper surface of the other very thoroughly with vaseline, and weigh. Hang the two leaves up again in the same place for four or more hours; weigh again and note loss of weight if any. The leaf vaselined on its under surface will show little loss in weight, while the loss from the other leaf will be comparable with that during the first two hours. It may be more, but for various reasons it may be less during the second and longer period.

108. Air Canals. The need for sealing the cut ends to prevent evaporation from that part will be quite clear, if another leaf be fixed in an air-tight manner into the stopper of a bottle of water so that the cut end of the leaf-stalk dips below the surface of the water and suction be applied to the air above the water by means of a glass tube attached to a filter-pump. When the suction is applied a continuous stream of air-bubbles is seen coming from the cut surface of the leaf-stalk, showing that there is a continuous air-passage from the external air through the stomata and down the leaf-stalk.

109. Pocket-lens observations on a thin slice of

rhododendron leaf-stalk will demonstrate these petiolar air-spaces quite clearly. *Ranunculus ficaria*, *Arum maculatum*, and many other leaves will show the same phenomenon.

110. *Loss of Weight.* That there is an actual loss of weight by the loss of water vapour has already been demonstrated in Ex. 107. This loss of weight can be followed over a long period, if a leafy plant in a small pot be arranged as in Ex. 105, and weighed at daily intervals. The plant should be well watered before commencing the experiment, which should be stopped whenever the leaves show signs of wilting or becoming flaccid.

111. *The loss of weight* from a known area of leaf surface should be compared with that by evaporation from a known area of free water surface. A shallow dish such as the half of a deep Petri capsule, should be used for the free water. The leaf surface is best attached to a living rooted plant ; for this purpose a small potted plant with more or less uniform leaves is most suitable, e.g. *Pelargonium*. The plant is arranged as in Ex. 105, and weighed at intervals of twenty-four hours. The transpiring area is then calculated as the total area of all the under-surfaces of the leaves, and the total loss in weight for each interval is reduced to loss in weight per square centimetre. The loss by evaporation in the case of free water is also reduced to loss per square centimetre for the same interval of time. If suitable potted plants are not available, freshly cut leafy twigs may be used for one period of twenty-four hours arranged in a Farmer's potometer (see below, Ex. 113.) The cut ends should be recut after each period and fresh initial weighings taken, when a severed leafy shoot is used.

112. The effects of structural arrangements for the control of transpiration are considered in Chapter XXIX, but *bloom* or the waxy covering of certain leaves can be removed, thus giving a good opportunity of experimentation. The most suitable material is furnished by well-grown carnations, but various species of *Echeveria* and *Crassula* are also suitable. In the case of carnations, a branch having a number of large leaves with well-developed bloom is removed carefully. Two similar leaves are cut off, the cut ends being sealed by dipping in melted hard paraffin. Care must be taken that no bloom is rubbed off. A thread is tied round each leaf, then they are weighed and hung up in a dry, dark place for twenty-four hours. The loss of weight is noted ; then the bloom is removed from one leaf with cotton-wool and warm water. After the surface of this leaf has dried at air temperature, both leaves are weighed and again hung up for twenty-four hours. The difference in loss of weight for the second period gives a measure of the protective controlling action of the "bloom."

ABSORPTION OF WATER BY LEAFY SHOOT

113. **Potometer.**¹ Set up a leafy shoot (which has been cut under water) in a Farmer's Potometer (see Fig. T 321). Determine the rate at which water is absorbed by the leafy shoot. The meniscus M should be brought to a point slightly beyond one of the main graduations of the scale, and the time taken for it to pass from that graduation to the next, say for 1 or 2 cm., should be noted. The meniscus should

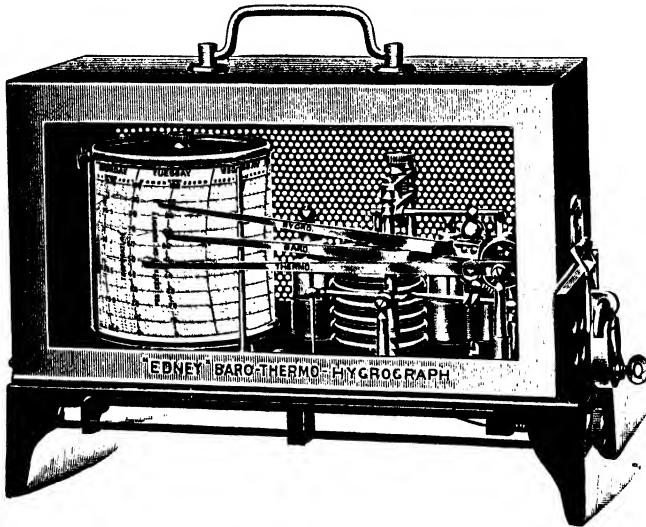


FIG. 9.—Edney Baro-Thermo-Hygrograph.

then be returned to the original point and the measurement repeated at least three times. The values obtained should not vary beyond narrow limits, showing that the rate of absorption under constant conditions varies but little.

The distance travelled by the meniscus in a given period of time, say, ten or fifteen minutes, may also be used as a measure of water-absorption, but, unless the same part of the capillary tube A is used for each time interval, the results are not as strictly comparable as in the first method, on account of variation in the internal diameter of the capillary tube.

¹ See Appendix II. for Potometer notes.

In these potometer experiments and all others where external conditions are effective factors, it is a great convenience to have an Edney Automatic-recording Baro-Thermo-Hygrograph in position. The record given by this instrument (Fig. 9) enables any effective variation during the experiment in barometric pressure, temperature or atmospheric humidity to be detected, and considered in relation to the results obtained.

Absorption and Transpiration.

114. Arrange a leafy twig and potometer as in Ex. 113, having previously calibrated the capillary tube so that you can calculate from the scale-readings the actual volume of water absorbed. Weigh the whole apparatus and place it in a warm, well-lighted situation with good ventilation. Take a series of scale-readings, returning the meniscus to the distal end of the tube after each period of absorption. Continue this series for two hours and calculate the total volume of water absorbed. Weigh the whole apparatus again. The change of weight in grammes is approximately the same as the water-absorption in cubic centimetres. *The rate of water-absorption can, therefore, be used as an approximate measure of the rate of actual transpiration or loss of water.*

Wind and Transpiration.

115. Arrange a leafy twig in a potometer as before and take notes of the rate of water-absorption until the absorption is stabilised and the rate becomes steady. Place an electric fan about 6 feet away from the apparatus, so that the current of air developed impinges upon the leaves. Note the consequent changes in the rate of transpiration as measured by the absorption rate. This may increase largely with a moderate current of air; it may increase only slightly, or even decrease, with a really strong wind, which produces secondary effects upon stomatal apertures.

116. **Light and Transpiration.** (a) Using the same arrangement as above, Ex. 115, expose a stabilised twig and potometer to direct sunlight. Note the rate of absorption until it is constant and then fix an opaque screen of three-ply wood or stiff cardboard so that the twig is shaded from one side only. The transpiration rate will show a distinct fall. Complete screening might alter the humidity conditions of the air around the leaf, but shading with a flat screen on only one side leaves these conditions unaltered and demonstrates the effect of light as such.

(b) Further darkening, giving more marked depressions, can be obtained by arranging two large glass plates at the sides of the potometer with a narrow roof joining the upper edges. The electric fan is then used to get a steady current of air through the tunnel. When this is secured, the twig can be exposed to sunlight under the tunnel or to very dim light by covering the glass with a large sheet of black paper. The Baro-Thermo-Hygrograph should be placed within the tunnel to check any variations of conditions.

117. An Artificial Twig. That transpiration is very similar to evaporation from a porous surface may be demonstrated by using an unglazed porous porcelain pot (about 8 cm. long and

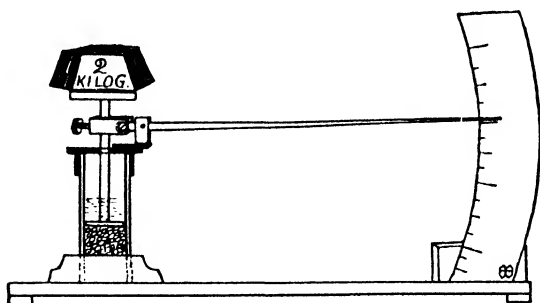


FIG. 10.—Imbibition Apparatus.

4 cm. diameter), attached by means of a rubber stopper to a 10 cm. length of glass tube, filled with water, and substituted for the leafy twig in the potometer arrangement of the above experiments. Using this apparatus as a modified atmometer,¹ a considerable degree of similarity may be demonstrated between evaporation and transpiration so far as wind action is concerned.

118. Imbibition. The internal evaporating surface of the leaf must be kept moist, and this involves the outward passage of water through the cell-walls against an osmotic or suction pressure. The force of imbibition which is brought in here as an explanation can be studied most readily using small seeds in an imbibition apparatus as figured (Fig. 10). This does not furnish quantitative data of the upper limits of imbibitional force, but the work done by a few mustard seeds in swelling as they imbibe water is sufficient to give

¹ See "The Plant in Relation to Water," by N. A. Maximov, London, 1929.

the student some idea of the average values. Actual results obtained by students vary from 88 to 172 gm. cm. for twenty-four hours with 1 gramme of seeds like those of the turnip and mustard.

119. (a) Higher imbibition values than those in Ex. 118 may be obtained by using a cylinder¹ of dry canary-wood or deal, inserted instead of seeds in the same apparatus, and substituting a 28 lb. weight for the 2 kg. weight which is used for seeds.

(b) That energy is freed during imbibition may be shown by stirring together dry starch powder and water with the bulb end of a thermometer. As the starch absorbs the water the temperature rises.

120. **Negative Pressure and Leaf-pull.** (a) Arrange a leafy shoot in a vertical glass tube with water over mercury (see Fig. T 319). Note the extent to which mercury rises. This measurement, which should be made in millimetres after an interval of one or two days gives evidence that the leafy shoot can exert a "positive pull" upon the water in the tube. This "pull" is correctly described as negative pressure.

(b) The corresponding pull of an artificial twig, see Ex. 117, should be determined. Its strength depends largely on the smallness of the pores in the pot used.

121. **The leaf-pull** brings the column of water in the stem into a condition of tension. The following experiment should be done on a warm dry day. Choose a flexible stem with leaves actively transpiring; young plants of broad bean or sunflower, or rather thin shoots of Jerusalem artichoke, are suitable. Keeping the plant with the roots attached to the stem, bend the stem below the surface of eosin solution in a flat dish. Keeping the part to be cut carefully submerged, cut the stem across with a sharp knife. The eosin solution is drawn into the cavities of the wood vessels in both directions, towards the root and towards the stem apex. The distribution of eosin staining should be examined and noted as a longitudinal diagram of each part of the plant. Microscopic preparations (combined with pocket-lens observations) should be used. The completed diagram will prove clearly that a leafy shoot shows negative pressure along the xylem of the stem.

A similar experiment with cacao-butter coloured with eosin yields a very interesting series of cross-sections after the fat has solidified in the cavities of the vessels. Tracheids are then

¹ This cylinder should be 2.5 cm. diameter and 4 cm. long and cut so that the grain runs with the thickness, not with the length.

distinguished from tracheæ by the different lengths which are filled with the coloured fat.

OSMOSIS

122. Thistle-funnel and Parchment. Arrange a parchment membrane and thistle-funnel with 10 per cent. sugar solution over water as in Fig. T 315. Take a note of the height of the sugar solution above the water-level every half-hour for as long a period as is suitable. This will depend on the membrane itself and on the way in which it is attached to the thistle-funnel.

A rise of solution in the stem of the thistle-funnel is explained as due to osmotic pressure (see Textbook, pp. 121–123). A subsequent fall may occur, which is explained by passage of sugar through the membrane and a consequent decrease in the *difference of concentration* on the two sides of the membrane. A slowing down of the initial *rate of rise* is explained by the dilution of the sugar solution as water passes through the membrane.

123. Traube's Cell. Prepare carefully a solution of potassium ferrocyanide by dissolving 1 gramme of that salt in 100 c.c. of distilled or soft tap water. Select a clean crystal of copper sulphate about 1 cm. long; attach the crystal to a support by a thin thread and suspend it *near the top* of the ferrocyanide solution in a *small* beaker. The successive solution of the copper sulphate, formation of a copper ferrocyanide membrane, the bursting of the cell so formed (with bluish contents and thin brown wall), the formation of a further membrane around the escaping internal solution, and so on leads to the development of a number of root-like structures. The process is slow enough to allow of observation of all stages and continuous enough to make the demonstration interesting for at least fifteen minutes.

Stronger solutions of ferrocyanide are not suitable for copper *sulphate*.

124. Diffusion and Osmosis. Prepare (1) a tall glass cylinder filled with water to within 5 cm. of the top, (2) a wide-bore tube with a parchment membrane and a beaker as for Ex. 122. Prepare also 200 c.c. of 10 per cent. copper sulphate solution and 100 c.c. of 50 per cent. sugar solution. With a pipette carefully introduce about 10 c.c. of the copper sulphate solution into the cylinder of water, so that the blue

solution remains at the bottom of the cylinder. Leave this quite undisturbed. Place the sugar solution in the wide-bore tube and immerse the parchment end in the remainder of the copper sulphate solution contained in a beaker of 250 c.c. capacity, supporting the wide-bore tube vertically. Compare the time taken for the blue colour to appear (1) in the upper layers of the fluid in the cylinder, where diffusion alone is acting, and (2) in the osmotic apparatus where osmosis and specific-gravity convection currents are acting. Protoplasmic circulation in the cell may take the place of these currents.

N.B.—The demonstration that the copper sulphate actually passes through the membrane only by diffusion and not by osmotic flow is an advanced problem of considerable complexity, and even research results of this kind are rare.

125. Osmotic Turgescence. In some of the lower plants, *e.g.* conidiophores of fungi, erect branches of sub-aerial algae, no mechanical supporting tissue is present and differential turgidity (see *Ex.* 126) is also absent. Erectness or rigidity then depends upon osmotic turgidity; the outward pressure of the internal solution against the inward pressure of the stretched containing wall gives a certain degree of rigidity. This phenomenon can be demonstrated by means of turgescient osmometers.

(a) A turgescient osmometer can be made by fitting a length of parchment tube with rubber stoppers, through one of which is placed a glass tube with a turn-tap and through the other a wide glass-tube with a small rubber stopper. The parchment has, however, a considerable rigidity, and osmotic turgescence is better shown by proceeding as follows :—

(b) Obtain a bottle of ordinary flexile collodion¹; clean a test-tube of at least 1.25 cm. diameter; when the test-tube is thoroughly dry, fill it with the collodion, then pour as much as possible back into the bottle, turning the test-tube as you pour. Set the tube aside for fifteen minutes or longer, until the collodion film inside ceases to be sticky; fill it with water and leave it for at least one hour immersed in water. The collodion film can then be separated very carefully from the glass-tube in the form of a long sac, by letting water from the tap run gently between the film and the inside of the test-tube. Prepare a piece of rubber pressure-tubing, about 3 cm. long and of such a thickness that it can be inserted easily into the test-tube. Pass the stem of a very small glass funnel through the stopper prepared in this

¹ This is supplied by retail chemists, and is the flexile collodion of the 1914 British Pharmacopœia.

way. Remove the collodion sac from the test-tube, place the top end around the rubber-stopper and tie it carefully with tape. Wire or thin string tends to cut the collodion.

Empty all water from the sac and pour in, by means of the funnel, sufficient of a 40 per cent. solution of cane sugar to make

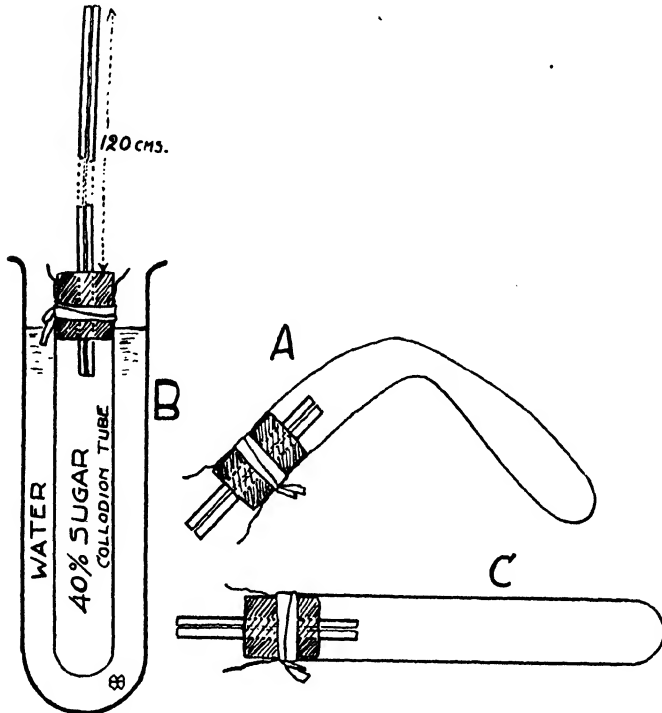


FIG. 11.—Turgescent Osmometer. A, Flaccid. B, In Water. C, Turgescent.

the sac about two-thirds full. The sugar solution should be previously coloured strongly with eosin or other dye. Replace the funnel by a piece of capillary-bore glass-tubing of similar outside dimensions and at least $1\frac{1}{2}$ metres long. Place the collodion sac in a wider tube, about 4 cm. diameter, filled with water and observe during half an hour (see Fig. 11). *Flexile* collodion is quite waterproof and nothing happens. Remove the sac from the water and dip it repeatedly into a wider tube of

95 per cent. alcohol, until the cloudiness disappears from the lower three-quarters of the collodion sac. The alcohol dissolves out the castor oil which makes the sac impermeable; and thus converts it into a semi-permeable membrane.

Replace the sac in the tube of water and make a series of observations. Note the entry of water which makes the sac full and quite turgid, so that it can support its own weight in a horizontal position (Fig. 11, A, C). Note the leakage of dye and sugar even while the coloured column is rising in the capillary tubing above. Note the maximum height reached in a few hours, followed by a gradual fall as the leakage brings about an equilibrium quickly on account of the small volume of outside water. Replace the first outside tube with another containing pure water and observe the consequent rise in the column (Fig. 11, B). Finally remove the capillary tube from the sac and try to blow the aqueous solution out of the narrow bore. This will give you some human idea of the force which has been exerted by the thin collodion sac, with its contents, in producing osmotic turgescence and in raising the column of solution many centimetres.

126. Observe the apparatus set up according to Ex. 125 as a demonstration experiment and take full notes concerning what you can see.

127. **Differential Turgidity.** The herbaceous stem of higher plants maintains its rigidity by means of a more elaborate mechanism than that demonstrated in Ex. 125. (a) Measure carefully a piece of Rhubarb petiole about 30 cm. long. Remove the whole of the skin, retaining the full length in at least two strips. Measure the length of the succulent core and the length of each of the two strips of skin. The rigidity of the stalk is due to the stretching of the skin and the contraction of the central tissues, while they are attached in their normal positions. The lengths, when these tissues are freed, depends in part upon the osmotic turgescence of Ex. 125.

(b) The cylinder of skin in the Rhubarb petiole is complete, but if this continuity be broken by cutting the petiole into longitudinal strips, the inner tissues will swell and the skin contract, so each strip curves outwards.

(c) The same curvature as in (b) occurs with many herbaceous stems cut at one end into four or more strips. The dandelion scape, the broad bean stem, the sunflower hypocotyl are all examples in which the curvature is increased by immersing the cut stems in water.

Plasmolysis

128. A turgescient osmometer prepared as in Ex. 125, but fitted with a solid glass tube as a stopper instead of a long capillary tube, can be made turgid by immersion in pure water or flaccid by immersion in the original strong sugar solution. In the flaccid condition it may be said to be "plasmolysed."

129. The term "**plasmolysis**" is more strictly applied to the cytoplasmic sac in the living cell. In the normal condition the cytoplasmic film is pressed against the cell wall, but, if the cell be immersed in a solution of higher osmotic strength than the cell sap, this film may be forced inwards away from the wall. The cell is then said to be "plasmolysed."

Any suitable material may be used, such as cells with coloured sap, *e.g.* beetroot, saxifrage epidermis. The material is mounted in water under a cover-slip, noted under the microscope and then irrigated with a strong osmotic solution, such as 20 per cent. cane sugar, or 5 per cent. sodium chloride or 5 per cent. potassium nitrate.

130. Deplasmolysis. In order to be sure that the cytoplasmic contraction observed is not due to injury and death, it is necessary to obtain a reversal of the phenomena observed in Ex. 129. If the cells are living but plasmolysed, the cytoplasm will recover its normal position when the material is transferred from the strong solution to plain water, by irrigation or otherwise. This process is known as deplasmolysis; a cell which can be plasmolysed and deplasmolysed is still alive.

131. Macroscopic observations should be made upon the apical 2 cm. of small roots or upon 4 cm. lengths of tissue cut from a rhubarb petiole. Such material becomes flaccid after immersion for several minutes in 5 per cent. potassium nitrate or sodium chloride. This is tissue plasmolysis. It recovers and becomes turgid again after a slightly longer time of immersion in plain water. The deplasmolysis or recovery shows that the tissue is still alive.

Measurement of Osmotic Pressure

132. The curvatures observed in Ex. 127 (c) can be utilised for an approximate measure of the osmotic pressure of the cell sap in the tissues concerned. Strips of dandelion scape or the hypocotyls of sunflower and *Ricinus* are used. These curl or

curve naturally. The dandelion scape is best because it yields spirals which can be cut until the normal curvature in air gives a complete circle, neither open nor overlapping. A ring placed in plain water curves more and overlaps; immersed in strong solution it curves less and the ring opens. An intermediate solution can be found, by a series of trials, in which the ring assumes the normal position in air, *i.e.* a complete ring, neither more nor less. The strength of this intermediate solution corresponds roughly in osmotic pressure to the “*effective osmotic pressure*” of the solution in the cells.

(a) Using dandelion scape as material and a series of solutions of KNO_3 ranging from 0.05 molar¹ to 0.20 molar, determine the “*effective osmotic pressure*” of the cell sap.

(b) Using material from the same source and a series of solutions of cane sugar ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$) ranging from 0.10 to 0.25 molar, determine the “*effective osmotic pressure*” of the cell sap.

133. Microscopic observations of the presence or absence of plasmolysis (see Ex. 129–130) may be used for the same purpose as the macroscopic method given above. The material should fulfil two conditions: (1) the tissue observed must not be injured to any significant degree; (2) the cells observed should have coloured sap so that any trace of separation of the cytoplasm from the wall may be seen. These conditions occur in easily stripped coloured epidermis and in many epidermal hairs.

(a) Using any suitable material and a series of solutions of cane sugar ranging from 0.10 to 0.25 molar, determine the “*effective osmotic pressure*” of the cell sap. In order to obtain an average value the number of definitely plasmolysed cells should be counted in a field covering about 50 cells. The critical strength of sugar solution is then that in which half of the cells are plasmolysed and the other half are not plasmolysed. A time factor may enter into the experiment, but observations after immersion of the tissue for twenty or thirty minutes should be used, the *same time* being allowed in each solution.

(b) Repeat your observations using similar material and a series of solutions of KNO_3 , ranging from 0.05 to 0.20 molar.

134. Isotonic Coefficients. Since the osmotic pressure of a substance in solution depends upon the number of ions present, it follows that a non-ionising substance like sucrose exerts a smaller osmotic pressure in solution than is exerted by a readily ionised substance such as potassium nitrate in the same molecular concentration. The results of Exs. 132–133 give molar concentrations which are comparable in their action upon tissues or cells, *i.e.* the molar concentrations found are isotonic with the

¹ Molar = gramme-molecules per litre, and is calculated from the molecular weight in grammes.

plant materials and therefore with each other. A quantitative expression is obtained if KNO_3 be taken as a unit and given the arbitrary value of 3.0¹; then from Exs. 132-133 we can calculate the Isotonic Coefficient of sucrose thus—

$$\frac{\text{Observed molar conc. KNO}_3}{\text{Obs. molar conc. sucrose}} = \frac{\text{I.C. sucrose}}{\text{I.C. KNO}_3} = \frac{\text{I.C. sucrose}}{3}$$

This value is slightly less than 2.0.²

Other methods of dealing with osmotic pressures and isotonic coefficients will be found in Chapter XXV.

SUCTION PRESSURE

The normal cell has an extensible elastic wall, which when distended exerts a certain inward pressure upon the contents, tending to squeeze out the water of the sap. The actual pressure or suction which draws or tends to draw water into the cell is therefore the resultant of (1) the effective osmotic pressure of the cell-contents tending to pull water in and (2) the pressure exerted by the distended wall (turgor pressure), which tends to squeeze water out. Briefly $S = P^e - W$, where S is the suction pressure, P^e is the effective osmotic pressure and W is the wall pressure. As this resultant suction pressure is normally different from the osmotic pressure of the sap and as it is the real factor which brings water into the plant, the student should know how the suction pressure of a tissue may be determined.

135. The Molz Method. The tissue to be investigated should be as nearly homogeneous as possible, *e.g.* epidermis peeled from a bulb-scale or part of the cortex or pith of a herbaceous stem. Potato tuber is not very suitable for this method, as applied by students. The tissue is placed in pure liquid paraffin for at least one hour, on a slide with a well and covered with a cover-slip. It can be left for several hours. Sections are made, if necessary, and short strips about 1 to 2 mm. in length are cut, and the length accurately measured under the microscope; see Ex. 392. The paraffin is removed gently by means of dry filter paper and the

¹ De Vries took as the original unit for isotonic coefficients one-third of the attraction for water of a molecule of potassium nitrate in dilute solution.

² A useful table of isotonic coefficients is readily available on p. 106 of "Permeability," by W. Stiles (N. P. Reprint, No. 18). Advanced students should at this point read carefully Chapter IX of that valuable treatise.

pieces are irrigated with cane sugar solutions of appropriate concentrations, e.g. 0.10 to 0.30 molar or stronger, and the slides with cover-slips enclosed in Petri capsules to prevent evaporation. The stock solutions should be stored in small *stoppered bottles* or tubes so that they may not become more concentrated by evaporation. The osmotic pressure of the sugar solution in which the length of the tissue strip remains unchanged corresponds to the suction pressure of the tissue. The following table includes a short range from the more extended data given by Molz (*Amer. Jour. Bot.*, 13, p. 433) :—

Molar Concentration. Sugar Solution.	Osmotic Pressure at 20° C. in Atmos.	Molar Concentration. Sugar Solution.	Osmotic Pressure at 20° C. in Atmos.
0.05	1.3	0.30	8.1
0.06	1.6	0.31	8.4
0.07	1.9	0.32	8.7
0.08	2.1	0.33	9.0
0.09	2.4	0.34	9.3
0.10	2.6	0.35	9.6
0.11	2.9	0.36	9.9
0.12	3.2	0.37	10.2
0.13	3.4	0.38	10.5
0.14	3.7	0.39	10.8
0.15	4.0	0.40	11.1
0.16	4.2	0.41	11.4
0.17	4.5	0.42	11.7
0.18	4.7	0.43	12.1
0.19	5.0	0.44	12.4
0.20	5.3	0.45	12.7
0.21	5.6	0.46	13.0
0.22	5.9	0.47	13.3
0.23	6.1	0.48	13.7
0.24	6.4	0.49	14.0
0.25	6.7	0.50	14.3
0.26	7.0	0.60	17.8
0.27	7.3	0.70	21.5
0.28	7.5	0.80	25.5
0.29	7.8	0.90	29.7

Further experiments will be found in Chapter XXV, see particularly Ex. 892.

PATH OF THE ASCENT OF SAP

136. Eosin. In Ex. 121 details are given of two methods by which the sap can be demonstrated as passing up the cavities of the vessels. Further work on these lines can be done. Choose a healthy herbaceous plant, such as groundsel (*Senecio vulgaris*) or cabbage seedlings; cut the root off near the top and stand the shoot in water coloured with eosin for several hours. Then cut a number of transverse and longitudinal sections from the base, the middle and the top of the stem. Near the upper limits of staining the eosin will be found only on the inner surface of the xylem elements.

137. Compression. Set up a potometer (Ex. 113) with a leafy woody shoot, e.g. privet branch. Determine the rate of absorption in the usual way. Now arrange the lower part of the branch in a small vice or between the two pieces of metal forming the inner part of an ordinary screw-clamp. The part of the stem to be compressed should be thickly covered with vaseline. Apply pressure by means of the screw until the potometer meniscus slows or ceases movement. Release the compression and note the recovery in absorption shown by the shoot.

The compression must be continued for a longer period to obtain wilting of the leaves and subsequent recovery (see Textbook, p. 127).

Passage across Root Cortex

The water in the xylem must have passed across the cortex of the root. This stage may be investigated by means of the following experiments.

138. Potato Osmometer. Choose an elongated potato tuber; cut each end flat and make a boring in the centre reaching more than half-way down (Fig. 12). Place this potato osmometer in a dish with sufficient water outside to reach a little above the level of the bottom of the hole inside. Pour into the hole a small quantity of 40 per cent. sugar solution and mark the level of the solution by sticking a pin into the tissue. Set aside for two hours and note the level again. It should be at least a centimetre above the original level. The actual rise can be measured by sticking another pin in at the second level, cutting the osmometer open and measuring from the first to the second pin.

indicating the presence of hydrogen. As the gases pass through the baryta water barium carbonate is precipitated, indicating the presence of carbon.

145. Tests for Nitrogen, Sulphur and Chlorine

Using about 0.5 gramme of the dry powdered material, heat gradually to redness in a test-tube with a piece of clean sodium metal. Plunge the hot tube into 50 c.c. of water contained in a porcelain basin of at least 200 c.c. capacity. Boil the resulting mixture of broken glass etc. gently, and filter off the aqueous extract. Repeat the extraction with 20 c.c. of water; filter and mix with the previous filtrate. In the aqueous extract—

Nitrogen may be detected thus: to 2 c.c. of the filtrate add one drop of caustic soda solution and five drops of a solution of ferrous sulphate and ferric chloride (0.5 per cent. each); boil; cool; acidify with hydrochloric acid. A blue colour, ferric ferrocyanide $\text{Fe}_4(\text{FeC}_6\text{N}_6)_3$, indicates the presence of nitrogen.

Sulphur may be detected thus: add a drop of lead acetate solution to 2 c.c. of caustic soda solution (1.0 per cent.); heat until clear; add this to 2 c.c. of the filtrate. A black precipitate of lead sulphide indicates the presence of sulphur.

Chlorine may be detected thus: acidify 2 c.c. of the filtrate strongly with nitric acid; boil for two minutes to destroy cyanides and sulphides; add a few drops of silver nitrate solution. A white precipitate of silver chloride indicates the presence of chlorine.

146. Test for Phosphorus. Melt in a porcelain crucible about 1 gramme of a mixture of equal parts of potassium carbonate and potassium nitrate; carefully add 0.1 gramme of dry powdered material; heat until effervescence ceases; cool; add 10 c.c. water; boil; filter; acidify with strong nitric acid; add ammonium molybdate solution and warm. A yellow precipitate of ammonium phosphomolybdate indicates the presence of phosphorus.

147. Heat a weighed quantity of the dried material in a crucible of known weight until all charring ceases and the residue is nearly white when cool. Weigh this residue and calculate the percentage of inorganic matter in the original fresh plant material.

Tests for Calcium, Potassium and Magnesium

148. This residue may be examined for calcium and potassium by holding a little of the substance in a Bunsen flame on a platinum wire.

Calcium gives a dull brick-red flame and **potassium** gives a violet flame which can be seen in the presence of calcium by looking through a piece of deep blue glass. The small spectroscope may also be used to see the bluish-green and orange lines of calcium together with the distinctive red and indigo-blue lines of potassium. **Magnesium** should be separated as magnesium ammonium phosphate in the usual way.

Essential Elements

The presence of the elements demonstrated in Exs. 144–148 does not mean that all of these are necessary for the healthy development of the plant. The effects produced by the lack of various elements can be investigated by means of water cultures or sand cultures. The former are more suitable for students' use, since sand cultures require more time and attention, both in preparation and in care afterwards, than the student is likely to have at his disposal.

149. Using such seedlings as may be available (*e.g.*, barley, wheat, pea, maize, sunflower), prepare eight water culture jars as follows :—

A. One litre containing the following pure salts :—

Calcium nitrate	1.0	gramme
Potassium nitrate	0.25	„
Magnesium sulphate	0.25	„
Potassium phosphate	0.25	„
Potassium chloride	0.12	„
Ferric chloride or ferrous phosphate	a trace	

B. Lacking nitrogen : 1 litre with sulphates substituted for nitrates.

C. Lacking phosphorus : 1 litre with chloride substituted for phosphate.

D. Lacking sulphur : 1 litre with phosphates substituted for sulphates.

E. Lacking calcium : 1 litre with potassium substituted for calcium.

F. Lacking potassium : 1 litre with magnesium and calcium substituted for potassium.

G. Lacking magnesium : 1 litre with calcium substituted for magnesium.

H. Lacking iron : 1 litre with all iron salts omitted.

Fit over each jar a cork cover with a support for the seedling, the roots of which are passed through a central hole in the cork. Fit also a piece of glass tubing through a second hole in the cork. Aerate the solution in each jar daily by blowing air through the glass tube. Observe and record the relative growth, development, and colour of the seedlings. The seedlings should be exposed to full daylight. Before completing the experiment, see Ex. 151, also Ex. 176.

150. Repeat the above experiment using the eight solutions A to H, in large Petri dishes, and beginning with ten similar plants of *Lemna* in each dish. Drawings and other records of the results should be made at least twice weekly for four weeks. In this

case the closed Petri capsules should be exposed to full daylight in a cool place. Aeration of the solutions is not necessary, if the lids are removed for one hour daily.

151. Selective Absorption. The careful quantitative methods which are required in order to follow the absorption by the plants of certain elements or ions in greater quantity than others is beyond the scope of this book. Some idea of the selection exercised may be obtained by comparing the chemical composition of an average soil with that of an average plant.

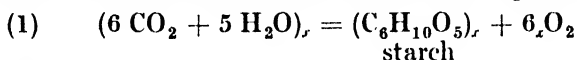
The student can, however, make an approximate analysis of the residual solution and deposit in the normal jar A of Ex. 149, using precipitation and gravimetric methods for calcium, magnesium, sulphate, phosphate, and chloride. If these values be subtracted from the total dry matter and the remainder assumed to be potassium nitrate, the selective absorption from the quantities originally supplied will be indicated roughly.

It should be noted that the use of certain substances rather than others in the building up of the plant here takes the place of the specific gravity convection currents observed in Ex. 124.

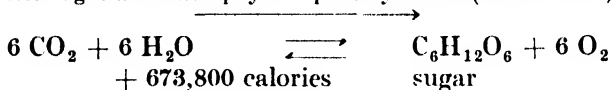
CHAPTER X

CARBON ASSIMILATION

THE first points to be investigated in the study of carbon assimilation are those involved in the two equations—



(2) With light and chlorophyll = photosynthesis (endothermic)



respiration (exothermic) = in dark with catalysts.

Since the starch of equation (1) is more readily made evident than the sugar of equation (2), the student should study the methods of detecting starch in green leaves.

STARCH TESTS

152. Sach's Iodine Method. Take a green leaf; dip it into boiling water for about one minute in order to kill the tissues and allow of easy extraction of the green substance. Immerse the leaf in alcohol until it is decolorised¹; then transfer the leaf to an aqueous solution of iodine and potassium iodide.² The blue-black colour of starch iodide indicates the presence of starch; this colour is discharged by strong alcohol, but reappears on dilution of the medium with water. The relative darkness of the coloration gives a rough measure of the amount of starch present.

153. Schimper's Method. Mount a few leaves of *Elodea*

¹ The alcohol may, if necessary, be warmed by placing the dish in hot water for twenty to sixty minutes or over a steam or hot water radiator, or on an electric stove, but heating with or near a naked flame is not recommended on account of the danger with student-manipulation.

² Iodine 1 per cent., KI 0.5 per cent. in water. This solution may be diluted.

or other small water-plant in Chloral Iodine,¹ and examine at intervals under the microscope. Note the clearing of the tissues and the distribution of the stained starch.

154. Starch-free Leaves. The development of starch under experimental conditions should be studied in leaves which are free from starch when the experiment is started. Using any suitable potted plants or jars with water plants, *e.g.* pelargonium or *Elodea*; place one plant as a control in the best light available and the other in the dark. Test one leaf from each plant for the presence of starch after 24, 48, 72, and 96 hours. The starch will be found to disappear almost or quite completely after 48 hours and quite completely after 96 hours.

155. Carbon Dioxide. (a) Connect up two wash-bottles containing baryta or lime water with a tube of soda-lime between the two bottles. Attach the chain to an aspirator or a slow-acting suction pump. Note the deposit in the first bottle and the absence of deposit in the second bottle. (b) Repeat with the first bottle detached. Test the deposit for carbonate with acetic or dilute hydrochloric acid. (c) Having proved by the above experiments (a) that carbon dioxide occurs in ordinary air and also (b) that air can be freed from its carbon dioxide by passing over soda-lime; fit up two wide-mouthed bottles with soda-lime tubes passing through rubber stoppers. Fill one of the tubes with soda-lime A; fill the other tube loosely with glass wool or leave it empty, B. Place in each bottle an equal but small quantity of water (about 2 to 5 c.c.) and six or eight small starch-free leaves,² *e.g.* of daisy, groundsel or pelargonium; expose both bottles to the best illumination possible. Remove one leaf from each bottle at intervals (i) of twenty-four hours, or (ii) of two hours on a bright sunny day. Test these leaves for starch and compare the results for the two bottles. This experiment proves that leaves do not produce starch in air free from carbon dioxide, and that they do produce starch if this and other conditions are present.³

¹ Chloral iodine may be made by dissolving five parts of chloral hydrate in two parts of water and adding crystals of iodine until a few remain undissolved. These should be retained in the bottle to maintain saturation.

² Leaves may be destarched by keeping leafy twigs (in water), or potted plants, in the dark for two days.

³ These experiments can be done during winter, but in frosty weather starch may not be formed.

Light

156. Place six or eight starch-free leaves, *e.g.* groundsel, daisy, in each of two wide-mouthed bottles with 2 to 5 c.c. water. Leave one bottle in a well-lighted place and the other in a dark cupboard. Test one leaf from each bottle at daily intervals.¹

157. Choose a plant with leaves having stomata on the under surface. The presence or absence of stomata on the upper surface is not a factor of importance in this experiment.

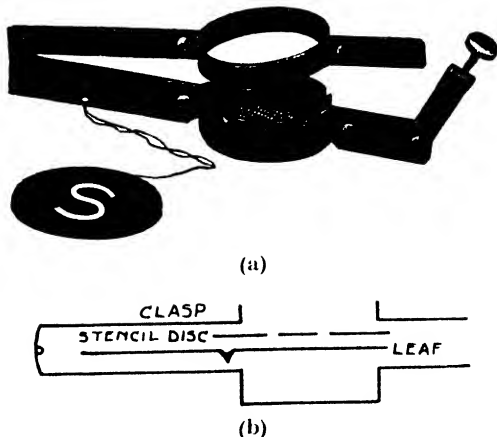


FIG. 13.—(a) Leaf-clasp, Type B with dark chamber below; (b) diagram to show position of clasp, stencil disc and leaf-blade.

Fix a leaf-clasp with a stencil cut from black paper² upon a leaf attached to the plant. Leave overnight so that any starch originally present may be removed. Remove the clasp and leaf after an exposure of at least four hours to good daylight has followed upon one night of darkness. Test the leaf for starch and compare the starch print with the stencil.

158. Chlorophyll. Choose a variegated leaf with as distinct a pattern as possible; remove this from the parent

¹ These experiments can be done during winter, but in frosty weather starch may not be formed.

² Leaf-clasp, Type B (Fig. 13), gives a good starch-print within twenty-four hours during the winter; the paper side of the disc should be placed next the leaf.

plant after it has been exposed to daylight for at least six hours. Make a drawing of the leaf to show the distribution

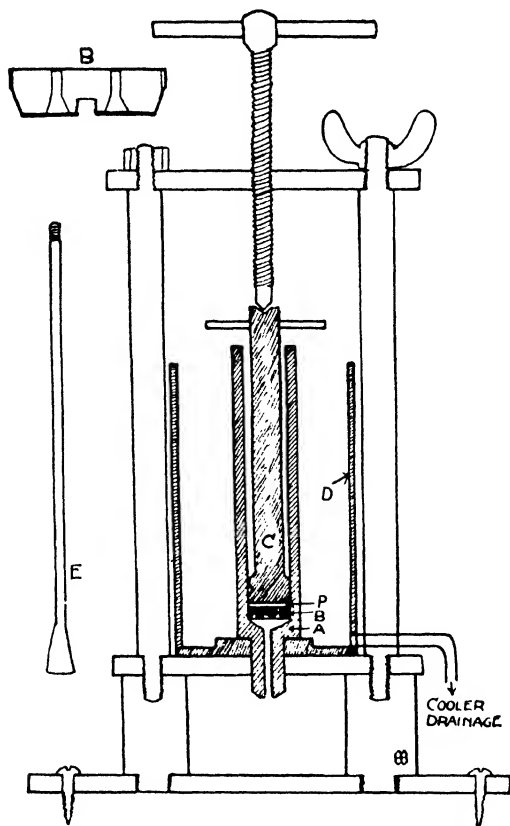


FIG. 14.—Small's Plant Press consists of A, a hollow cylinder, B, a grooved disc and C, a plunger, surrounded by D, a copper vessel for ice-cooling. The plant material is pressed at P, between the disc and the plunger by the screw-press. The parts A., B. and C. are made of non-corroding metal. E. is an enlarged view of a tapping screw to remove the disc for washing after use.

of green and yellow parts ; then decolorise the leaf and test for starch. Compare the starch-pattern obtained with the plan of colour distribution.

OTHER TESTS

159. Sugar. According to the second equation (p. 70), sugar is produced; and the above experiments are all based upon the production of starch. The production of sugars should be investigated by the more advanced student. Use a potted plant; water this well and keep it in the dark for at least four days. Remove two or three leaves and press out the juice in a small plant press (Fig. 14). Filter the juice and to one-half add Fehling's solution. If there is no red precipitate when this is boiled, heat the other half of the juice with dilute sulphuric acid for ten minutes, neutralise, add Fehling's solution and heat again. The absence of sugars will be indicated by no reduction of the Fehling's Solution in either case. Using sugar-free leaves of this type, expose them for one hour to bright daylight; repeat the extraction of juice and test as before for sugars. The results should now be positive.

160. Oxygen. The production of oxygen is most easily shown in water-plants. Set up a beaker, funnel and test-tube with water and *Elodea* in good daylight (see Textbook, Fig. T 322). Collect at least half a tube of gas and test for oxygen by the usual methods.

161. Volume Relations. According to both equations, the amount of oxygen given off is equal to the amount of carbon dioxide taken in, and within an enclosed space this should result in no change of pressure. Spread a few healthy green leaves over the bottom of a flask so that they can be all well illuminated and attach the flask in an air-tight manner to a mercury manometer. Set the apparatus in a well-lighted situation and note the mercury levels after two, four, and six hours' illumination.

Methods of Estimating Photosynthesis

162. Thoday's Method. This depends on measuring the increase in dry weight of a fixed area. The leaves, which should be reasonably large, are stamped in ink with a rubber stamp. A rectangle 3 cm. by 1.5 cm. has been found convenient. These areas should be stamped in equal numbers on each leaf, choosing parts of the leaf-blade relatively free from large veins. After at least sixty such areas have been stamped, those on one side of each leaf should be removed and trimmed along the middle of the inked line. The trimmed pieces of leaf are then dried below 100° C. until the weight is constant. After the other thirty areas have been exposed to the experimental conditions for a known period of time they are removed, trimmed accurately

and dried as before to a constant weight. The difference in the dry weights should be recorded as a measure of the rate of photosynthesis, calculated to the terms "per hour per square decimetre of leaf surface."

163. **Oxygen Method.** Choose a shoot of *Elodea* or other convenient water-plant, fix it in position under water with the freshly cut basal end up, but completely submerged. Bubbles of gas may be seen coming from the cut end, and the number of these per minute gives a rough measure of the rate of photosynthesis under varying conditions. The accuracy of this method may be increased by fitting a small glass tube, drawn to an open

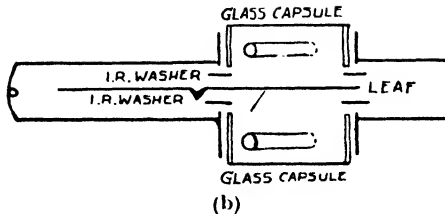
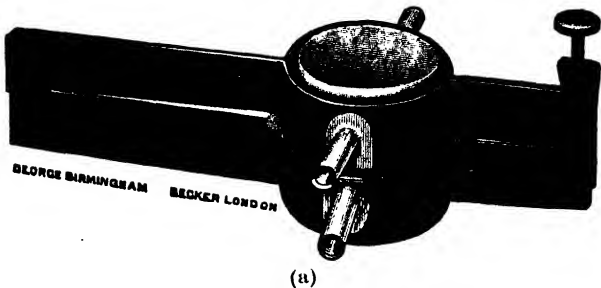


FIG. 15.—Leaf-clasp, Type D. (a) General view. (b) Diagram showing position of leaf.

point, over the cut end of the shoot. The number of bubbles per minute should be determined for bright light and at least two stages of duller illumination, using a Watkins' photographic exposure meter or other actinometer to obtain a measure of the light intensity in each case. For convenience in manipulation the "glass bubbler" may be tied to a small glass tripod or a coil of glass-tubing; the cut end of the shoot is then inserted into the supported bubbler.

164. **Gas Analysis Method.** The gaseous exchanges of the leaf may be investigated by slowly passing a known volume of a

definite mixture of gases over the leaf surfaces¹ and analysing the mixture after it has passed over the leaf. Carbon dioxide may be absorbed completely by standardised alkali either in a Pettenkoffer tube or in a bottle fitted with a Jena glass gas-distributing tube (patterns 33c G¹ or 83 G¹). For approximate determinations it is sufficient to pass the gases through two wash-bottles containing baryta water, preferably fitted with gas distributing tubes, collect and weigh the precipitate as barium carbonate or titrate back with N/10 HCl in the usual way (see Ex. 200.). Oxygen may be determined by absorption from a definite volume of the gas by leaving this in contact with an alkaline solution of pyrogallate, which, of course, absorbs the carbon dioxide in addition to the oxygen. For accurate work a Haldane's Gas Analysis Apparatus may be used.

Leaf-clasp, Type D (Fig. 15), has been designed to make convenient the enclosure of the leaf surfaces on the living plant. Two glass capsules and rubber washers are applied to the upper and lower surfaces of the leaf. The capsules and washers are sealed to the leaf by means of a mixture of equal parts of soft paraffin and beeswax. Air containing about 2 per cent. carbon dioxide can then be passed through a T-tube over each leaf surface under the experimental conditions, and one of the various methods for absorption of carbon dioxide used to obtain a measure of the gaseous exchange of *each leaf surface*.

CARBON DIOXIDE

165. Diffusion of Carbon Dioxide. Take a tall graduated glass jar; lead a little of the gas from a carbon dioxide Kipp's apparatus gently to the bottom of the empty jar by means of a glass tube, and leave the cylinder undisturbed for five minutes. Observe the slow upward diffusion of the carbon dioxide, by noting the graduation at which a clear drop of lime or baryta water becomes milky when it is slowly lowered into the cylinder.

Diffusion through Stomata

166. That carbon dioxide enters the leaf through the stomata may be demonstrated, roughly, by using the Starch-Iodine Test (Ex. 152) on a leaf with one half of the under side covered with vaseline to block the pores and the other half left free.

¹ The gas mixture prepared in a Winchester quart bottle may be passed over the leaf slowly by allowing water to drip into the bottle from a stoppered funnel fixed through the cork of the bottle. The volume of water allowed in from the funnel should be about 500 c.c. per hour; see Ex. 171.

167. Using starch-free leaves with stomata on the under surface only, apply three Leaf-clasps, Type A, to the leaves thus : (a) use both discs so that both surfaces are blocked ; (b) use one disc only on the upper surface ; (c) use one disc only on the under surface. Expose the three leaves to bright light for four hours ; apply the Starch-Iodine Test and compare the results obtained ; (a) and (c) will be similar, (b) will show normal starch content because the under surface is left free.

168. Using leaves as above (Ex. 167) apply Leaf-clasp, Type D. as in Ex. 164 and compare the weights of barium carbonate obtained when the leaf has been illuminated by bright daylight. All the carbon dioxide supplied is recovered from the upper surface, but there is a measurable loss in the case of the under surface.

169. Advanced students should repeat the above experiment, using a wash-bottle of strong sulphuric acid before the T-tube leading to the leaf-chambers, so that the leaf is gradually brought into a condition with dry-shut stomata on the under surface.

170. **Dixon's Diffusion Experiment.**¹ Choose five specimen tubes about 1.5 cm. wide and about 4 or 5 cm. deep ; smear the rims with sticky Canada balsam ; place about 4 c.c. of petrol in each tube and arrange a pair of microscope cover-slips over the top, so that a slit with parallel edges is left for outward diffusion of the petrol vapour. The slits should be of the following approximate widths : 3 mm., 1 mm., 0.5 mm., 0.25 mm., and 0.125 mm. Set the tubes of petrol aside IN A SAFE PLACE, well away from any naked flame ; weigh them when ready to be set aside and at daily intervals for one week afterwards. Tabulate for each tube the weight lost and the length, width, margin, and area of the slit. Convert these figures into easily comparable data by taking tube No. 1 in each column as 100. This demonstrates clearly that the marginal length, or the width with constant length, is the determining factor in gaseous diffusion through a slit such as a stomatal opening.

171. Carbon Dioxide as a Limiting Factor

(a) Using Leaf-clasp, Type D, as in Ex. 164 and a known volume of N/10 Na OH, either in a Pettenkoffer tube or in a washing-tower fitted with a gas-distributor, determine the amount of carbon dioxide absorbed by a green leaf in direct sunlight when 0.1 per cent. carbon dioxide is used. Your results may show that the well-illuminated green leaf can use up more than 0.1 per cent., if the gas is passed at the rate of 500 c.c. per hour. The rate can be adjusted by graduating the bottle of gas, and allowing an *even* flow of water from the funnel so that 500 c.c. of water

¹ See *Nature*, 18/10/30.

enters the bottle in one hour, or 500 c.c. may be poured into a large funnel and allowed into the bottle at a steady rate.

(b) Using two similar sets of ten well-illuminated starch-free leaves of moderate size, contained in two wide bottles each fitted with two tubes, pass 3 litres of 0.03 per cent. carbon dioxide (air) through one bottle in six hours and 3 litres of 1.0 per cent. carbon dioxide through the other bottle during the same time. Compare the dry weights of the two sets of leaves and note the greater production of material by the second set of leaves.

N.B. The leaves should be arranged inside the bottle in such a way that *each leaf* is reasonably well-lighted and *no leaf* is mainly covered.

172. Toxic Effects of Excess. (a) Attach two Leaf-clasps, Type D, to starch-free leaves upon a potted plant; connect the exit-tubes of upper and lower capsules by a short length of rubber tubing; place the enclosed leaf surfaces so that both are well-lighted; during the next six hours pass a steady stream of air through one clasp and a similar stream of 50 per cent. carbon dioxide through the other. Two Winchester quart gas-bottles may be used as in Ex. 171 (a). Test for starch in both leaves and compare the enclosed areas with the free, unshaded areas. The absence of starch from the second enclosed area indicates a toxic effect of the high concentration of carbon dioxide.

(b) Leave two sets of leafy shoots of *Elodea* or *Callitriche* in the light for several days, both submerged in water, one set exposed to ordinary air under an inverted beaker and the other exposed to 50 per cent. carbon dioxide displaced into a similar inverted beaker. Apply Sach's Iodine Test after not less than four days. A toxic effect is again indicated.

LIGHT

173. Etiolation. Set about sixty white mustard seeds or thirty green peas to germinate and grow into seedlings in darkness, in three pots of well-moistened soil. All three pots should be carefully shielded from daylight until they are ready; examination of their growth can be made by means of a dull red light. When the seedlings are from 2 to 3 inches long, place one pot in the brightest daylight available and take notes every fifteen minutes for three hours of the changes in colour which follow the development of chlorophyll. Place a second pot in a shaded place indoors; note the slower development of chlorophyll; test the green leaves for starch and observe that the light may be strong enough for the appearance of chlorophyll, but too weak for the production of starch. Leave the third pot in darkness until

the others are used, and then expose it to bright daylight for one daily period of twenty minutes or for two daily periods of ten minutes; replace it in darkness and repeat the exposure to light daily for at least one week. Observe the result of brief exposures to light. See also Ex. 175.

174. Similar experiments may be carried out with broad beans, runner beans (*Phaseolus multiflorus*), sprouting potatoes, pelargonium plants and young shoots of *Polygonum cuspidatum*.¹

Seedlings of *Pinus* produce chlorophyll in the dark. They require about four weeks for germination. Sycamore (*Acer*) cotyledons are quite green and remain so during germination of these seeds in the dark.

175. **Oxygen and Chlorophyll Formation.** A few etiolated seedlings from one of the pots in Ex. 173 should be used to demonstrate the effect of lack of oxygen on chlorophyll development. Two or three seedlings should be exposed to light in a closed Petri dish with a little water, and another two or three in a similar dish completely filled with water so that there is no layer of air above the seedling. These dishes left for twenty-four hours show a very clear difference in the contained seedlings.

176. **Chlorosis.** Using the plants from pot H of Ex. 149, or other available chlorotic material, test the effect of iron on the yellow leaves. Chlorophyll may develop in iron-starved leaves when the roots are immersed in water containing 0.1 per cent. ferric chloride, or when the leaves are painted with 0.01 per cent. ferric chloride solution. If these tests fail, the internal hydrogen concentration of the mesophyll may be less than pH 5.8 and the iron therefore not available, although present outside the cells. This condition can usually be modified by placing the etiolated plants in an atmosphere containing 20 per cent. carbon dioxide, while applying iron solution to the roots. Another method consists of centrifuging a single seedling immersed in a test tube of 1 per cent. citric acid solution to get injection of the tissues, and following this treatment with the external application of 0.1 per cent. ferric chloride.

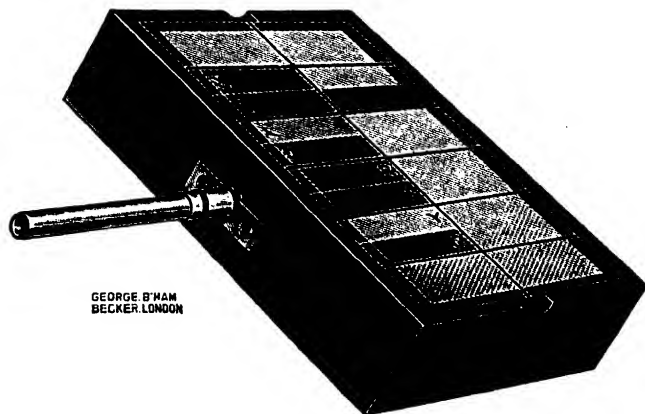
Light Intensity

177. Using a potted plant with large starch-free leaves and a leaf-screen box (see Fig. 16 below), expose one leaf to continuous daylight for at least eight hours under a

¹ Cp. pp. 287 *sqq.* in "Recent Advances in Plant Physiology," by Barton Wright, also Priestley in *The New Phytologist*, XXIV, No. 5, 1925.

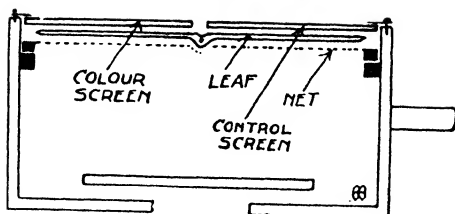
photographic negative with strong contrasts or under a Dremmeter.¹ "Develop" the leaf by Sachs' Iodine Method, Ex. 152, and note the varying depths of grey in the resulting starch print.

178. Using starch-free leaves in wide-mouthed bottles



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(a)



(b)

FIG. 16.—Leaf-screen. (a) General view. (b) Diagram.

with a little water, expose three sets of leaves to (a) dull indoor light, (b) shade outdoors, (c) full daylight for at least eight successive hours. Test each set of leaves for starch in the late afternoon and note the varying depths of tone in the results. This gives a striking demonstration of the effect of shade, even outdoor, upon starch formation.

¹ The Dremmeter is a film negative with definitely graded densities in a number of small rectangles. It can be obtained from Messrs. Sands, Hunter & Co.

179. Colour Tests. Using leaves attached to the plant, expose leaves under colour-screens of different colours for at least two hours of bright daylight after one night in the dark.¹ Test the leaves for starch and compare the densities of the resulting starch-stains with the densities marked upon the Dremmeter scale.

(a) **Leaf-clasp Type C** (Fig. 15) has been designed to make this experiment somewhat more accurate. The coloured celluloid discs have the same transmission spectra as the corresponding colours of the Leaf-screen (see below). It is therefore possible to compare the starch-stains qualitatively and, by using the Dremmeter scale, quantitatively with some degree of accuracy.

(b) **Leaf-screen.** This is a box with provision for ventilating the under surface of the leaf in relative darkness while exposing the leaf, flattened against a net, under screens of various kinds (Fig. 16). The photographic negative or Dremmeter is used in Ex. 177. The colour screen with strips of red, blue, yellow and green should be used in the present experiment and the results under these strips compared with those given by white light and under the black control strip. The transmission spectra are given in Fig. 17; and these should be noted carefully, since the yellow and green screens transmit a certain percentage of red light-rays as well as the more obvious colours. The effect of the red and blue screens can be compared freely; their visual luminosities are about equal, but their energy transmissions differ markedly, so do the resulting starch prints.

Dr. K. G. Emel us and Mr. R. H. Sloane report upon this colour screen as follows :

A logarithmic sector was rotated in front of the slit of the laboratory medium glass spectrograph, and photographs of the spectrum of an opal bulb taken with and without filters, the light being passed through the latter normally. The Spectra were photographed on Agfa "Special Rapid Panchromatic" Plates and Kodak "Extreme Red Sensitive" plates. Neon and Copper arc lines were used as standards, and the percentage transmission at each wave-length taken to be

$$100 \times \frac{\text{Wedge length of spectrum with filter}}{\text{Wedge length of spectrum without filter}}$$

¹ Potted plants should be kept in the dark for twenty-four hours before being used. Outdoor leaves should be carefully tied up in brown paper bags the evening before they are required for the experiment. Starch stains can be obtained after *eight* hours even on a dull winter day, if starch-free leaves are used for this experiment.

This formula is not exact (*cf.* Hilger's catalogue), but is probably exact enough for the present purpose. Taking Langley's curve for the distribution of energy in sunlight, as quoted in Pfeffer's "Physiology of Plants," the accompanying transmission graphs were prepared, and the total energy transmission between 4,000A and 7,500A found by integration of the areas beneath the curves.

The following points should be noted :

(1) There is considerable energy transmitted beyond the infra-red limit of the present investigation ; this would be most conveniently found, if necessary, by a bolometric or calorimetric method.

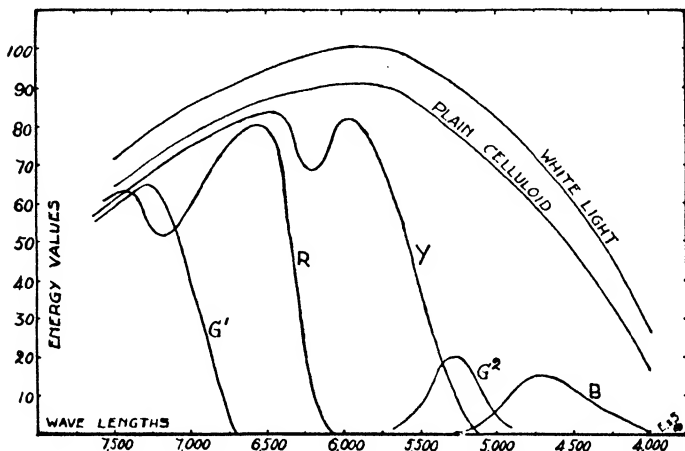


FIG. 17.—Energy Transmission Curves of Colour Screens used in Leaf-clasp, Type C. and Leaf-screen. R. red, Y. yellow, B. blue screen transmission curves, G¹ and G² green screen transmission curve. The areas below the curves give by simple inspection a rough idea of the relative amounts of energy transmitted. Compare the report on p. 81. (After Emeléus and Sloane.)

(2) For work where high accuracy is aimed at, the transmission curves furnished should be redetermined by better methods, *e.g.* at the N.P.L.

Percentage of total energy transmitted between 4,000A and 7,500A :

Plain celluloid	88 per cent.
Blue filter	3.5 ..
Green	15 ..
Yellow	58 ..
Red	28 ..

The experimental leaf should be kept attached to the plant and be comparatively large, *e.g.* sunflower, wild beet, etc. The leaf is spread upon the net and the two screens fixed in position above. One screen transmits white light only, with one section completely darkened; this acts upon one half of the leaf as a control. The colour sections of the other screen are arranged so that the sequence from the base of the leaf upwards can be reversed. The Leaf-screen gives a more convincing demonstration than that of Leaf-clasp, Type C, of the effects of light from different parts of the spectrum, because the *same leaf* is exposed under different screens and the results, especially when checked by using the reversed sequence of colours on a second leaf, cannot be attributed to variation in the activity or health of the leaf.

180. Light as a Limiting Factor. (a) Prepare a healthy shoot of *Elodea* for bubble-counting as in Ex. 163. Arrange three 60 watt electric lamps in a row and place the beaker of water, with a thermometer in it and with the *Elodea*, about 50 cm. away from the central lamp. With one lamp lighted count the number of bubbles per minute at intervals over a period of twenty minutes; repeat with three lamps lighted; repeat with only two lamps lighted. Check the temperature, which should remain practically the same throughout the experiment. Take the average number of bubbles per minute under the three conditions of light and compare these values with the relative intensities of illumination. The water should be at room-temperature when the experiment is commenced. Movement of the beaker should be avoided, but a more uncertain variation in intensity may also be obtained by moving a single lamp. See Exs. 182 and 183.

(b) Set up a Leaf-clasp, Type D, a gas supply of 1.0 per cent. carbon dioxide, and a gas analysis apparatus as in Ex. 171. Illuminate the enclosed surface of green-leaf as in Ex. 180 (a) and compare the rate of absorption of carbon dioxide under three intensities of light over periods of at least twenty minutes. The flow of gas supplied keeps the temperatures within narrow limits.

181. Toxic Effects of Excess. Prepare a heat-screen by placing a deep Petri dish containing a saturated solution of alum over a detached leaf lying with its stalk in a little water. Focus direct sunlight upon the leaf surface by means of a reading-lens or other concave lens of long focus. Note the comparatively rapid browning, indicating decomposition of the chlorophyll by intense illumination.

Check the efficiency of the heat-screen by substituting a thermometer for the browned part of the leaf.

TEMPERATURE

182. The effect of temperature variations may be studied conveniently with the apparatus of Ex. 180 (a). Using two or three lamps to get a reasonable number of bubbles per minute from the *Elodea* shoot; prepare two further beakers of water, one (A) kept at about 5°C . by means of a small piece of ice, and the other (B) at about 25°C .¹ Transfer the *Elodea* shoot and "bubbler" from the original beaker to B and note the bubbling rate until it is reasonably constant; then transfer the plant and bubbler to A and again take a note of the number (if any) of bubbles per minute. The evolution of gas may cease on transferring to A. If this happens write out a complete list of the possible causes; cessation of photosynthesis may not be the actual cause of the stoppage.

183. Temperature as a Limiting Factor. The above experiment may be extended by using only one 60-watt lamp at a distance of 25 cm. from the outside of the beaker and starting with a temperature below 5°C . The beaker with its contents should be placed upon an asbestos ring at least 1 cm. thick, above an electric hot-plate and the temperature raised very slowly by *intermittent very short* periods of heating, switching the hot-plate on and off as required. When the number of bubbles per minute remains the same for three successively higher temperature changes, a second lamp should be added and the observations repeated. In this way the combined effects of light and temperature may be studied. See also Ex. 184.

184. Toxic Effects of Excess. (a) By means of the electric hot plate, the temperature can be raised gradually and the toxic effects of temperatures higher than 30°C . made quite evident.

(b) Using the normal hot-plate observe carefully under the high power of the microscope the behaviour and appearance of chloroplasts in a leaf of *Elodea* when the temperature is gradually raised above 30°C .

TRANSLOCATION

185. Disappearance of Starch. Place a healthy plant, e.g. potted pelargonium or *Elodea*, in darkness and make a

¹ The temperature may be kept up by the addition of hot water or by means of *intermittent and very short* periods of heating on an electric hot-plate. Thermometers should be retained in both beakers.

starch test on one or two leaves each morning until no further starch stain is obtained. Examine small portions of the leaves daily by Schimper's Method, Ex. 153.

186. More advanced students should be practically acquainted with quantitative sugar methods ; see Mangham, *New Phytologist*, 10, p. 160, 1911 ; also *Annals of Botany*, 29, p. 369, 1915 ; Mason and Maskell, *Memoirs Cotton Res. Stn.*, Trinidad, 1928 ; and for the Multiple Razor as a means of examining different parts of the same leaf, Small, *New Phytologist*, 15, p. 194, 1916.

PROPERTIES OF CHLOROPHYLL

Materials. Nettle leaves—these may be collected during the spring or autumn and dried rapidly at a temperature below 40° C., by suspension in a large muslin bag over a radiator or in other ways. When brittle-dry these leaves are easily powdered. After further drying at 40° C. for three days, this powder keeps well if stored in containers which prevent the access of moisture, *i.e.* well-filled stoppered bottles, or jars fitted with quick-lime desiccators such as are used for keeping biscuits.

Alcoholic alkali. This is a 30 per cent. solution of potassium hydroxide in *methyl* alcohol.

187. **Acetone Extract.** Place 5 grammes of powdered leaf in a wide-mouthed stoppered bottle of 100 c.c. capacity ; add 48 c.c. anhydrous acetone and 12 c.c. water ; set aside for twenty-four hours, shaking occasionally ; filter through paper and use suction to obtain as much filtrate as possible ; at least 40 c.c. should be obtained ; transfer this acetone solution of the pigments to a narrow-mouthed well-stoppered bottle of brown glass.

188. **Dichroism.** Pour a little of the acetone extract into a watch-glass, observe the blue-green colour by transmitted light over white paper. Then look obliquely through the layer of solution and note the deep red colour by reflected light. This dichroism is characteristic of the crystalline condition of the green pigments. Return the liquid to the bottle.

189. **Colloidal Chlorophyll.** Take up 5 c.c. of acetone extract in a pipette and allow it to flow into 50 c.c. of water in a small beaker, stirring continuously with the pipette. Note the slight change to a less bluish tint of green. Transfer some of this colloidal solution to a small flat-sided bottle ; examine and map the spectrum (see Fig. T 325, also Ex. 190, below).

190. Action of Light. Fill another small flat-sided bottle with acetone extract and place both this and the bottle of fluid used for spectrum observations (Ex. 189) in bright daylight. Observe, compare and note the colour changes during twenty-four hours.

191. Two Green Pigments. Pour 10 c.c. of acetone extract into 20 c.c. of petrol ether contained in a separating funnel; add *carefully* 30 c.c. of water. Set aside until a deeper green ethereal layer separates; run off the slightly green aqueous layer. Wash the petrol ether solution of mixed pigments gently with two successive quantities of 20 c.c. of water, separating and running off the aqueous layer completely. If the petrol ether solution is turbid add 2 grammes of exsiccated sodium sulphate, shake and filter. Add the petrol ether solution to an equal volume of 92 per cent. *methyl* alcohol; shake and set aside. Two green layers separate out; the upper petrol ether layer contains chlorophyll *a* and carotin, while the lower methyl alcohol layer contains chlorophyll *b* and xanthophyll. Compare the spectra of the two solutions with Fig. T 325.

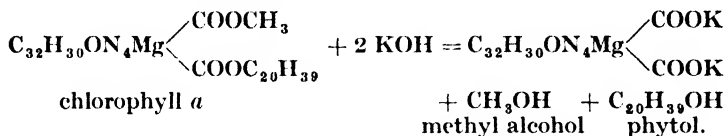
192. Separation of Green and Yellow Pigments. Pour 10 c.c. of acetone extract into 30 c.c. of ether, add water, separate, and wash the ethereal layer as for petrol ether in the previous experiment. Shake 4 c.c. of "alcoholic alkali" with 10 c.c. of the ether solution¹ of mixed pigments, until a green colour develops. Add slowly 20 c.c. of water and then 5 c.c. of ether; shake gently. Two layers separate, an aqueous lower layer containing both green pigments saponified by the alkali and an ethereal upper layer containing both yellow pigments (see Ex. 193, below).

193. Two Yellow Pigments. Run off the aqueous layer of Ex. 192, and wash the ethereal layer gently with three successive quantities of 20 c.c. water. Evaporate the ethereal solution, in a shallow dish out-of-doors, until the volume is about 2 c.c. Transfer to a separating funnel; add 10 c.c. petrol ether and shake; then add 10 c.c. 90 per cent. methyl alcohol and shake. On standing, two yellow layers separate; the lower alcoholic layer contains only xanthophyll, the upper petrol ether layer contains all the carotin and some xanthophyll. The remaining xanthophyll may be washed out by repeating the washing with fresh

¹ The remainder of this original ether solution should be reserved for Exs. 194, 195, and 196.

quantities of 10 c.c. 90 per cent. methyl alcohol until the alcoholic layer is no longer coloured. The yellow petrol ether then contains only carotin. Examine the spectra of the two solutions and compare them with Fig. T 325.

194. Action of Alkali. Chlorophyll *a* is the ester of methyl and phytol (primary) alcohols with the dicarboxylic acid chlorophyllin; it is saponified by the action of alkali in the cold to an alkali salt of the acid, somewhat as follows:



Take about 3 c.c. of the original "ether solution" from Ex. 192 in a test-tube, introduce 5 c.c. of *alcoholic alkali* as a lower layer by means of a pipette. Note the brown phase which develops at the junction of the two layers. Shake gently; the brown colour disappears and the fluid changes gradually from olive-green to pure green as saponification is completed. See Onslow's "Plant Biochemistry" for details of the reactions.

195. Action of Acids. The chlorophylls are changed by the action of acids, with the removal of magnesium from the molecule: chlorophyll *a* gives phæophytin *a*; chlorophyllides give the corresponding phæophorbides (see Onslow, p. 35; or Willstätter and Stoll in "Untersuch. ü. Chlorophyll").

Take about 3 c.c. of the "original ether solution" from Ex. 192 in a test-tube, introduce 5 c.c. of dilute hydrochloric acid as a lower layer by means of a pipette. Note the brown phase which develops at the junction of the two layers. Shake gently; the brown colour disappears and the fluid changes to a muddy bluish-green with precipitation of some of the phæophytin.

196. Action of Copper Salts. Place about 0.1 gramme of copper acetate in a test-tube; add four drops of water and then six drops of strong hydrochloric acid, using a pipette or a dropping bottle in each case. Shake and, when solution is complete, add carefully 2 c.c. of the "original ether solution" from Ex. 192. Shake gently and note that the colour changes to a more brilliant green. Add 5 c.c. water and evaporate the ether on a water bath with care. Cool and transfer a part of the solution to a watch-glass; examine for dichroism. The substituted copper compound is not dichroic (cp. Ex. 188).

197. Action of Chlorophyllase. (a) This enzyme is particularly abundant in fresh leaves of *Heracleum sphondylium* and *Galeopsis tetrahit*. Cut fresh leaves of one of these species into small pieces; pack 1 gramme of comminuted leaf in a test-tube and add 4 c.c. of 70 per cent. hydrous acetone. Set aside for at least half

an hour, with occasional shaking. Add about 15 c.c. water and transfer to a separating funnel; add 3 c.c. ether, shake, separate and collect the ethereal layer in a test-tube. Add to this 10 c.c. of 0.05 per cent. potassium hydroxide and shake gently. The chlorophyll, which is insoluble in weak aqueous alkali, is hydrolysed by the *chlorophyllase* to phytol and acid-chlorophyllide (soluble in ether), the latter reacts with dilute alkali to give a chlorophyllin salt soluble in water and insoluble in ether.

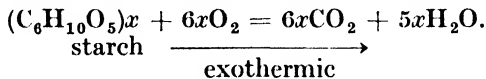
(b) That this is an *enzymic hydrolysis* may be shown by boiling some fresh leaves of the same species in water for ten minutes, extracting with hydrous acetone and proceeding as before. The unaltered chlorophyll does not react with the weak aqueous alkali, which does not, therefore, extract the colouring matter from the ethereal layer.

Many further experiments and theoretical details will be found in "Practical Plant Biochemistry," by M. W. Onslow, Cambridge University Press. Advanced students are advised to study the chemistry of chlorophyll by means of that volume. The above experiments are suitable only for first- or second-year students.

CHAPTER XI

RESPIRATION

NORMAL respiration, represented in summary fashion as the oxidation of carbohydrate materials with the liberation of energy and the production of carbon dioxide, may be presented as the familiar equation—



198. Carbon Dioxide. The production of carbon dioxide by any part of the plant may be demonstrated roughly thus : Fit rubber stoppers to six wide-mouthed bottles ; place 5 c.c. water in each bottle and cover one bottle A to the neck with black or thick brown paper. Place some green leaves in this bottle A ; a similar set of leaves in an undarkened bottle B ; some moistened seeds in another C ; some opening flower- or leaf-buds in a fourth bottle D ; the root parts of two groundsel or other similar plants in a fifth E ; and leave the sixth F as a control. Set all six bottles in a well-illuminated position for about twenty-four hours. After a period of at least four hours of bright daylight, take a lighted taper ; ease the rubber stopper gently from each bottle in turn and test the ability of the gas-content to support combustion. Note the results for each bottle and write a brief explanation of each observation. Compare A and B with particular reference to carbon assimilation in the light.

199. Sachs' Experiment. Set up the apparatus as given in Fig. T 326. Attach the last bottle, of baryta water, to a suction pump or aspirator and note the result.

200. The above experiment can be made quantitative by using two absorbing bottles ¹ of baryta water after the bottle containing the respiring material. The amount of carbon

¹ These should be fitted with gas distributors such as the Jena glass types 33c G¹ or 83 G¹.

dioxide given off by a noted quantity of plant material in a noted time may then be determined either by weighing the precipitate as barium carbonate or by using a known quantity of baryta water and rapidly titrating 20 c.c. of the mixed contents of both bottles with N/10 HCl, using methyl red or methyl orange as an indicator. A check titration with 20 c.c. of the original baryta water gives by calculation the amount of carbon dioxide absorbed by 20 c.c. of the absorbing fluid, and from this the amount absorbed by the total volume can be determined. If x be the total volume in cubic centimetres of baryta water used, and y be the *difference* in cubic centimetres of N/10 HCl used for the two lots of 20 c.c.,

then, at 15° C., $1.17y \times \frac{x}{20}$ or $.0585xy$ is the total volume in cubic centimetres of carbon dioxide given out during the experiment.

201. Respiroscope. One of the many types of respiroscope may be used for enclosing moist seeds or other respiring material in the upper bulbous part of the bent tube, the lower end of which may be placed simply in a solution of alkali when a considerable rise is obtained, or in mercury with the introduction of alkali on the mercury surface within the vertical part of the tube when a small rise is obtained which enables the experiment to be used as a demonstration over a much longer period.

202. Respirometer. Various modifications of the simple respiroscope are used to measure the rate of respiration. These replace the continuous gas stream of Sachs' Method, Exs. 199–200, by a closed volume of air used for a short period. This enclosure in a space containing an increasing percentage of carbon dioxide may have an effect upon the rate of respiration. The usual type of respirometer with a small plant-chamber is especially liable to this error, but it can be used by advanced students who understand the factors. The large flask used in Ex. 205 is not so likely to give trouble or wrong results.

203. Starch. (a) That starch disappears from leaves *attached to the plant* is demonstrated in Exs. 154 and 185, but the accepted explanation lies in translocation of the starch in the form of soluble sugars. Remove eight leaves from a plant; make a starch test of one leaf at once; place the others in the dark with moist cotton-wool *not* in contact with the leaves, and make a starch test on one leaf each day

for several days. Note that the disappearance of the starch cannot be due to translocation, but it might be due to conversion of the starch into sugars.

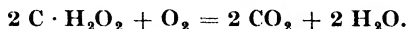
(b) Take about 20 grammes of leaves from the same plant ; divide them into two lots of exactly equal weight ; determine the dry weight of one lot as soon as possible by drying at 96° C. until the weight is constant, and of the other lot after the period of time shown by the above experiment to be required for the complete disappearance of starch. The difference in the dry weight indicates that some at least of the starch has been converted into volatile products such as carbon dioxide and water vapour. This experiment may be extended as in Ex. 206.

204. Heat. Set up **Potter's Experiment** (see Textbook, pp. 151-152 and Fig. T 327). Graph the temperatures daily for three weeks and write an explanation of the curves obtained. For crude experiments, designed merely to demonstrate that there is some heat given off in respiration, Potter's apparatus may be replaced by three chalk-boxes or three single-walled round-bottomed flasks, but each box or flask should have a drainage hole in the bottom for carbon dioxide.

205. Respiratory Quotient. Set up the apparatus as figured (Fig. 18), consulting the Textbook, pp. 158-159, for details.¹ Determine the R.Q. of starchy seeds such as cereals ; oily seeds such as white mustard, turnip, or rape ; sugary scale leaves such as onion ; and small entire leaves of succulents such as *Sedum* spp. Using about fifty white mustard seeds suitable results can be obtained after two hours' respiration. Peas may be used as an instructive study of an abnormally high R.Q. In this case the R.Q. may be 1.2, and more carbon dioxide is given off than the oxygen taken in, whether the seed is freed from the seed-coats or not. Two processes are concerned, namely : (1) peroxidase reactions in which organic peroxides with water give hydrogen peroxide capable of yielding an extra atom of oxygen for carbo-

¹ The present apparatus is an improved version of Fig. T 328 (2nd edition). The improvements consist of the Easy-reading R. Q. Gauge with its levelling tube N and the graduations arranged to give more accurate readings I and II where the difference is small ; also the normal thermostat F in place of an improvisation ; and the glass rod E inside the bulb C. This rod E is used first to scratch the inside of the bulb C and then, by a slight tap, manipulated by means of the longer rubber tube at D, the alkali is liberated into the flask B. Instead of using a thermostat, the total volume of the flask and its connections may be determined, and the gauge readings, as variations of this, reduced to N.T.P.

hydrate oxidation and twice the normal volume of carbon dioxide, thus :



(2) Zymase reactions in which zymase reacts with sugars with the production of carbon dioxide and alcohol as in yeast-sugar zymasis, thus :



The first process is the main disturbing factor when a large flask and aerobic conditions are used ; the second process is dominant under the anaerobic conditions of Ex. 209 below.

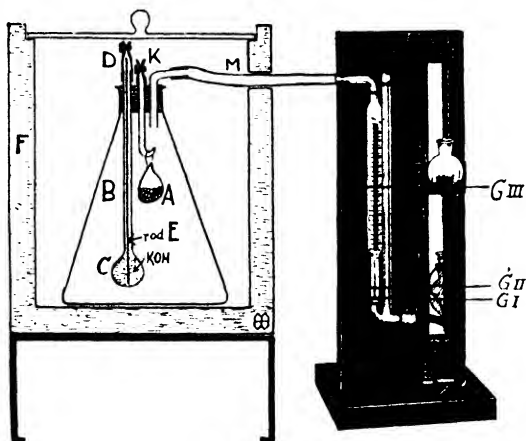


FIG. 18.—R.Q. Apparatus, see text.

Normal respiration is dominant under the conditions of the present experiment, but peroxidase action is always present to some extent in moist peas under aerobic conditions, see Chapter XXX.

Advanced methods of determining the R.Q. under conditions of a continuous gas-stream are numerous and varied, but outside the scope of an elementary class-book such as this.

206. Changes in Materials. (a) **Leaves.** Repeat Ex. 203 (b) and determine, in addition to the dry weight of each lot of leaves, the proportion of water-soluble matter in each lot, by macerating the dried and weighed leaves with three successive quantities of 50 c.c. water ; collecting, drying and weighing the insoluble residue.

(b) **Seeds.** The determination of increase in water-soluble matter may also be made using seeds, one lot being moistened

and kept in the dark for at least two weeks, before the dry weight and water-soluble matter is determined, for comparison with the same data concerning an equal weight of the original seeds, dried at 96° C. until they cease to lose weight (see Ex. 203 (b)).

(c) **Succulents.** Oxidation of carbohydrates in succulents usually proceeds only to the intermediate stage of organic acid production, without the liberation of carbon dioxide.

(i.) The consequential intake of oxygen without the liberation of a corresponding volume of carbon dioxide may be demonstrated by fixing a few small leaves of a succulent,¹ e.g. *Sedum* spp., *Bryophyllum calycinum*, *Rochea falcata*, etc. with glass-wool, or loosely packed cotton-wool, in the upper part of a simple test-tube inverted in water. A considerable rise of the water into the tube may be observed the following morning.

(ii.) This experiment may be made more precise by using a known weight of leaves¹ in a darkened flask attached to the R.Q. Gauge. The actual acidity or *pH* may be examined before and after the experiment, using small sections of the succulent mesophyll and the R.I.M.² The total acidity of a known weight of control leaves and of the experimental lot after a night in darkness may be determined, by freezing the comminuted leaves, expressing the juice (see Ex. 159), and titrating this fluid with N/50 potassium hydroxide, using thymol-blue or phenolphthalein as an indicator. Both the actual acidity and the total acidity show an increase in successful experiments.

207. Starvation. Place a few moist seeds or leaves with 2 c.c. water, and a short test-tube or small bottle containing 10 c.c. 50 per cent. potassium hydroxide not in contact with the plant material, in a flask. Place the flask in a darkened thermostat at 20° C. or 25° C. and connect it with the R.Q. Gauge. Measure, at atmospheric pressure, the volume of carbon dioxide absorbed by the alkali, *i.e.* the difference in the gauge readings, daily at the same hour for two or three weeks. The flask may be opened daily to bring the gauge reading back to about zero. Graph your results and write an explanation (see Textbook, p. 155). Instead of using a thermostat the total volume of the flask and connections

¹ In order to make sure of a good result, this experiment should be done with leaves which are taken from the plant about 5 p.m. after a day of bright sunshine and used immediately.

² See Chapter VIII. "Hydrogen-ion Concentration in Plant Cells and Tissues," Small, Borntreger, 1929; also Chapter XXV. below.

may be determined and the gauge readings, as variations of this, reduced to total volume at N.T.P., but in that case the experiment cannot be extended as in Ex. 211.

Anaerobic Respiration

208. Fit the rubber stopper of a small conical flask with a glass tube bent twice, thus Γ , with the short arm through the rubber stopper. Place in the flask a small quantity of yeast mixed with sufficient of a 10 per cent. sucrose solution to fill the flask within 2 inches of the neck. Fix the stopper firmly to get an air-tight container. Arrange the long arm so that it dips into baryta water or lime water contained in an adjacent bottle which should be fitted with a two-hole rubber stopper. The bent tube fits through one of the holes and a filled soda-lime tube is fitted into the other hole to protect the carbon-dioxide absorbent from atmospheric contamination. Set aside for several hours. Note the evolution of bubbles of gas from the yeast-sugar mixture. After a period, depending upon the activity of the yeast, a sufficient pressure will be developed to give a bubbling of the evolved gas into the baryta water, with a resulting precipitation of barium carbonate. Frothing of the yeasty fluid may occur, but the experiment should be completed before this froth overflows. On opening the flask, alcohol may be detected by its characteristic odour.¹

209. Fill a test-tube with mercury and invert it over mercury in a trough. Support the inverted tube with a clamp-stand, a little above the bottom of the trough. Insert four moistened and skinned green peas under the edge of the tube. They float up to the top and respire by zymasis, with the gradual production of carbon dioxide giving a lowering of the mercury, and of alcohol which may be detected by the odour.¹

210. Viability of Seeds. (a) See Ex. 22, which should be carried out at this stage, if omitted earlier. The seeds of willow or cypselæ of Coltsfoot (*Tussilago farfara*) may be used.

(b) Determine the germination capacity of the white mustard or other seed from a stock bottle, upon which is entered the results of similar experiments in previous years. Graph the data given and add your own results.

¹ Small quantities of alcohol may also be detected by distilling the mixed fluid, collecting 2 c.c. of distillate; warming this with one or two small crystals of iodine, and adding N/10 potassium hydroxide drop by drop until the brown colour disappears. The characteristic odour of iodoform develops, if alcohol is present in the distillate.

daylight and the other in darkness or strong shade. After the seedlings are several weeks old, cut the shoots into small pieces ; freeze these and extract the juice in a plant-press (Fig. 14). Evaporate this juice to a small volume on a water-bath ; transfer to a test-tube and add half its volume of a 40 per cent. solution of potassium hydroxide. Boil the mixture and examine the vapour for ammonia by means of red litmus paper and a glass rod dipped in strong hydrochloric acid. The etiolated plants should give the reaction readily, while the juice from green plants may not yield ammonia at all.

NITROGEN FIXATION

216. Tubercles. Wash the roots of a well-grown bean or pea plant in running water. Examine and sketch the distribution of tubercles. Draw several tubercles on a larger scale to show the variations in size and shape. Cut thin sections of one tubercle, double stain and make permanent preparations. Draw a line diagram showing the location of the cells containing bacteria, and also representative parts of the section on a larger scale (Fig. T 330).

217. Cycas. Examine and draw a prepared transverse section of the root of *Cycas* ; a line diagram of the whole section and a detailed drawing of a small part of the algal zone should be made. Note under H.P. the bacteria within the cells as well as in the intercellular spaces of the algal zone (see Textbook, p. 162 ; also Spratt in "Annals of Botany," 29, 1915).

218. Varieties of Tubercles. Examine all the available material of root-tubercles, and note the variations in appearance, structure and morphology. Note particularly the modified lateral roots of *Alnus*, *Myrica*, and other non-leguminous plants with peripheral bacteroid zones and central vascular tissue, as compared with the leguminous nodules of external origin which have central bacteroid regions and peripheral vascular strands.

The nodules of leguminous plants may be grouped as four types, thus --

I. Genistæ type, with spherical meristem, a broad basal zone of vascular tissue and several masses of bacteroid tissue, e.g. *Lupinus*, *Genista*.

II. Phaseolæ and Trifoliæ type, with apical meristem, one basal vascular strand branching 1-4, and one central mass of bacteroid tissue, e.g. *Trifolium*, *Ononis*, *Phaseolus*.

III. Viceæ type, with well-defined apical meristem and two separate vascular strands, e.g. *Vicia*, *Pisum*, *Lathyrus*.

IV. Mimosoideæ type, tubercles persisting for more than one year. See Spratt in "Annals of Botany," 32, p. 189, 1919.

219. Comparative Cultures. Prepare three 4-inch flower-pots by filling them with well-washed sand and heating them, when filled, for three hours in a dry oven slightly above 100° C. Allow the pots to cool within the oven; meanwhile prepare nine young seedlings of bean or pea by removing with a sharp knife the cotyledons and all roots which have root nodules appearing on them. Plant three seedlings in each pot A, B and C. Water pot A with a full water-culture solution; water B with a similar culture solution lacking nitrates; water pot C with a culture solution lacking nitrates, but mixed with about 2 grammes of ordinary garden soil. See Ex. 149 for culture solutions. Take notes of the condition of the seedlings weekly for two months. At the end of that period examine and record the degree of development of tubercles in pot A with nitrates supplied, in pot B deprived of both nitrates and bacteria, and in pot C deprived of nitrates, but supplied with bacteria.

220. Soil Bacteria. Obtain a small "clean" sample of soil by taking about 10 grammes from the centre of a spadeful; add this to 250 c.c. of tap water which has been boiled and cooled, with a plug of cotton-wool to prevent contamination. Break up the soil sample in the water with a sterile glass rod and mix well. Draw off 10 c.c. of the supernatant liquid and transfer this to a second flask of 250 c.c. sterile water; mix; repeat this process twice. Inoculate a Petri dish of nutrient agar¹ with 1 c.c. of the liquid from the fourth flask and incubate the plate at 20° C. Examine the preparation daily without opening the Petri dish. Count the colonies and note their form and colour. When the culture is one or two weeks old some of the bacteria may be identified by comparison with the figures in Waksman's "Principles of Soil Microbiology." Plates opposite pp. 8, 72, 108, 122, 142, 158, 160, 192.

Some of the chief forms connected with the nitrogen-cycle may be recognised thus:

Rhizobium (= *Pseudomonas* or *Bacillus*) *radicicola*: variable but, in culture, motile and ellipsoidal, about 1 micron long; also as minute rods, slightly larger cocci, longer rods and bacteroids (Fig. T 330); old colonies white.

Azotobacter chroococcum: usually non-motile, elliptical, usually 3-6 microns long and 2-3 microns wide, but variable and occurring

¹ Nutrient agar may be prepared as follows: to 1 litre of distilled water, add in grammes 1.0 K_2HPO_4 , 0.1 $MgSO_4 \cdot 7H_2O$, 0.1 $CaCl_2$, 0.1 $NaCl$, 0.5 KNO_3 , 0.5 asparagin, a trace of ferric chloride; dissolve and add 15 grammes agar; filter when hot and add 1.0 gramme mannite. Adjust the reaction of the medium to pH 7.4. See Waksman for other formulæ.

also in sarcina and filamentous forms; old colonies yellowish-brown to brown.

Clostridium pastorianum (= *C. butyricum*, *Bacillus amylobacter*, etc.): anaerobic; multiciliate; straight, cylindrical, usually 2-3 microns long, single or in pairs or chains; spores usually at one end; old colonies acid to litmus.

Granulobacter pectinovorum, anaerobic; forms variable, with spore at one end; now regarded as synonymous with *Clostridium butyricum*, *Granulobacter butylicum*, etc.

Bacillus asterosporus: aerobic; multiciliate, rods 3-6 microns long, single or in pairs; spores usually excentric, stellate in end view; colonies raised, transparent, white to yellow; fixes atmospheric nitrogen.

Nitrosomonas europaea, oblong, up to 1.8 microns long, with polar flagellum three or four times as long as the rod when motile; does not grow well on organic media.

Nitrobacter winogradskyi, non-motile, rods about 1 micron long, with pointed non-staining ends; does not grow well on organic media.

INSECTIVOROUS PLANTS

These should, as far as possible, be collected in their natural habitats and examined as material becomes available.

Drosera

221. Note the axillary scales or squamulae, the petiolar marginal hairs and the stalked glands or tentacles of various lengths. If the plants are in flower, note also the small anthers and elongated seeds (see "Pocket-lens Plant Lore," Small, Churchill, No. 119).

222. Cut part of the white of a hard-boiled egg into millimetre cubes and place one on each of several active, bright red tentacles, preferably belonging to different leaves.¹ Examine these daily and note the gradual rounding of the cubes, which are ultimately reduced to small fluid spots. Similar cubes exposed on the wet moss show little or no change in the same period.

223. (a) Place a piece of raw meat about the size of a pin-head upon one of the bright red outer tentacles of a young fresh *Drosera* leaf. Watch the head of the tentacle under a dissecting microscope or a firmly fixed pocket-lens of short focus. Movement may be detected within thirty seconds and strong curvature in seven or eight minutes.

(b) Repeat, placing pieces of meat in different positions with relation to the centre of the leaf, and note that inflection is directed towards the stimulated region.

¹ The plants for Exs. 222-226 may be kept alive in very wet moss in a shallow dish. They should be fully illuminated and well aired.

224. Repeat the above experiment using fragments of broken porcelain or coloured glass. Inflection occurs, but is considerably slower.

225. Remove six healthy leaves from the plants by cutting the base of the petiole; immerse three leaves in distilled water and the other three in water mixed with a little chalk. Note the difference after ten or fifteen minutes. The chalky fluid stimulates the leaves to inflection by the repeated contacts of chalk granules

with the sensitive glandular heads of the tentacles. A single touch, or repeated touches with drops of water, will not produce stimulation.

226. Prepare a 1 per cent. solution of ammonium phosphate; dilute 2 c.c. of this to 1 litre with distilled water; this gives a concentration of 1 in 50,000. Set four leaves in a shallow dish of this solution and four control leaves in a shallow dish of distilled water. After one or two hours it will be seen that stimulation is possible even with such a dilute nitrogenous fluid.

227. *Pinguicula*. Note and draw the leaves in various stages of stimulation. Observe the remains of insects on older leaves. Cut a thick cross-slice of a leaf and examine a side view

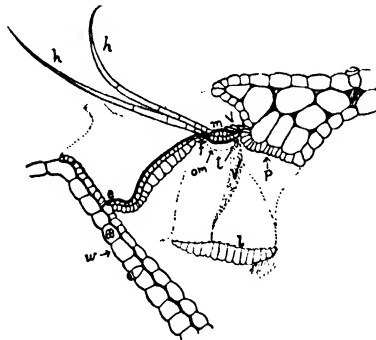


FIG. 19.—*Utricularia* door-apparatus: *hh*. trip hairs; *m*. door-edge or flange shut; *om*. door-edge in trip position before passing to full opening; *v*. cuticular fold acting as veil or valve, derived from and attached to *t*. the threshold or upper part of *p*. the pad; *e*. hinge region; *f*. thin fulcrum region which bends to give *om*. position; *v'*. lateral part of veil; *l*. lateral attachment of door; *w*. back wall of bladder (modified after Lloyd).

of this under the highest power of a dissecting microscope or a $1\frac{1}{2}$ inch objective. Note and draw the glandular hairs.

228. *Dionæa*. Make a clear line-drawing of one complete leaf. Note the apical position of the trap apparatus and the angle made by the teeth with the flat surface. Observe the trigger-hairs and, if sufficient material be available, cut a small piece from this flat part and observe under L.P. the multicellular sessile glands (Fig. T 333).

229. Pitcher Plants

(a) *Nepenthes*. Draw a complete leaf to show the stalk, the flat lamina, the tendril stalk of the pitcher, the pitcher (Fig. T 334) with its lid and minute spike at the hinge. This spike is

the true tip of the leaf. Draw also a median longitudinal elevation of a pitcher. Examine the inner surface of cut pieces of pitchers, under L.P., for nectaries and crescent-shaped secretory glands.

(b) **Other Pitchers.** Examine and draw single leaves of fresh or museum specimens of *Sarracenia*, *Darlingtonia* and *Cephalotus*.

230. Utricularia. Examine a living plant, floating in a deep Petri half-capsule, under a dissecting microscope. Draw a few bladders to show the collapsed and distended conditions. Using a dissecting microscope with the highest magnification available and a pair of sharp-pointed mounted needles, hold a bladder carefully and raise the door to see the flexible flange. With a sharp knife cut the door portion from a distended bladder; mount it in water; examine and draw the hairs upon the upper anterior surface of the door and other details as far as they can be seen under L.P. (Fig. 19). Cut a bladder in pieces, and examine the inner surface under H.P.; note and draw the stellate hairs (Fig. T 336.)¹

231. Lathraea. Examine and draw the leaves of the material supplied. Look for the minute opening on the underside of a leaf. Cut a thin section of a leaf; make a permanent preparation. Draw a line diagram of your section and, under H.P., a representative portion of the inner surface of the labyrinth to show a few of the scutate and capitate kinds of secretory glands.

For further details and illustrations see Darwin's "Insectivorous Plants," Kerner and Oliver's "Natural History of Plants," Haberlandt's "Physiological Plant Anatomy," also M. C. Cooke's "Freaks and Marvels of Plant Life."

¹ The correct interpretation of this mechanism is given by Professor F. E. Lloyd in "Plant Physiology," Vol. 4, No. 1, 1929, where the flexible flange, the valve and the lever action of the "hairs" upon the door are described.

CHAPTER XIII

BUDS

232. Examine a complete herbaceous plant or the twig of a shrub ; note the **axillary buds** and the **apical buds** either on the main shoot or on the branches. Make a diagram to show the positions of *both* kinds of buds in relation to the stem and to the leaves or leaf-scars.

Adventitious Buds

233. (a) Examine the preparations of leaves and roots showing the development of buds which are neither axillary nor apical. Make line drawings on a large scale to show the place of origin of these buds, *e.g.* *Bryophyllum* leaves, rose roots.

(b) Using the simple germinator (Fig. 5), or other means of keeping the material moist, set aside for some time slices of fresh root of dandelion or dock or plantain and either two young leaves of *Bryophyllum calycinum* or two leaves of *Cardamine amara* or other weed-cress. The last may be cut into as many pieces as there are pairs of lobes on the leaf. Note the times taken for the development of (i.) adventitious buds, and (ii.) adventitious roots (*cp.* Ex. 93).

(c) Prepare a shallow earthenware dish of well-washed sand, moistened with tap-water. Cut a series of slices $\frac{1}{8}$ to $\frac{1}{16}$ inch thick from a daffodil or tulip bulb, and place these flat on the surface of the sand. Cover the slices with a Petri half-capsule raised above the sand at one side by a short length of glass tubing. Keep the sand moist, but not wet, and note the development of adventitious bulblets. This is a crude modification of the commercial process for rapid multiplication of bulbs.

234. Accessory Buds. Examine and sketch the prepared specimens of accessory buds, *e.g.* potato tuber, sycamore, walnut, lilac and *Fuchsia* stems.

Winter Buds of Biennials

235. Remove the leaves from the Brussels sprout supplied, carefully, one at a time. Lay them out in sequence from

base to apex. Continue the process, using dissecting needles, until the apex is reached (as in autumn) or until a group of flower-buds appears, like a cluster of minute drumsticks (as in spring). Draw three leaves to show the large, medium and very small types. Make a large-scale drawing of the axis of the bud to show (i.) the scars left by the leaves removed, and (ii.) the axillary buds. Cut a longitudinal slice of these axillary buds and note the conducting strands of leaf-base and bud. For illustrations see "Pocket-lens Plant Lore," Pl. 21, No. 168.

236. Compare with the Brussels sprout the structure of the cabbage and its axillary buds (see "P-L. P. L.," Pl. 3, No. 19), also the structure of a small part of the white heart of a cauliflower (*Ibid.*, Pl. 21, No. 167). Make line diagrams to show the comparative development of stem, leaves, axillary buds, and flower-buds in these three forms of *Brassica oleracea*.

237. Outdoor Observations. Note the other buds which occur in biennials. Compare the apical buds of wallflower or snapdragon with the one-year-old rosettes of foxglove and mullein.

238. Winter Buds of Water-plants. Examine and sketch the prepared specimens of winter-buds of hydrophytes, e.g. *Hydrocharis*, *Hippuris*, *Utricularia*.

239. Winter Buds of Trees. Examine twigs of the following trees: lime, sycamore, horse-chestnut. Make an accurate drawing of each, showing (i.) bud-scales, (ii.) young foliage leaves, (iii.) bud-scale scars, (iv.) lenticels, (v.) dormant or active axillary buds, (vi.) old foliage leaf-scars. Locate in your drawings the annual increment of growth in each case. Dissect buds from two of the twigs and note the nature of the bud-scales.

240. The winter buds and leaf-scars of trees are frequently very characteristic. Many of these are illustrated and commented upon in "Pocket-lens Plant Lore"; Plate IV. of this is reproduced here as Fig. 20.¹

Fig. 20 includes: groundsel, with compound flower-buds; white willow, with two leaf-blades fused to form one bud-scale; privet, with an inner bud of leaf-blades, which

¹ Further details may be found in the above-mentioned book, or in "Buds and Stipules," by Avebury, published by Kegan Paul, etc., London.

BUDS

emerges from two scales ; oak, elm and birch, with stipular bud-scales ; *Aucuba*, with leaf-base bud-scale, also a second

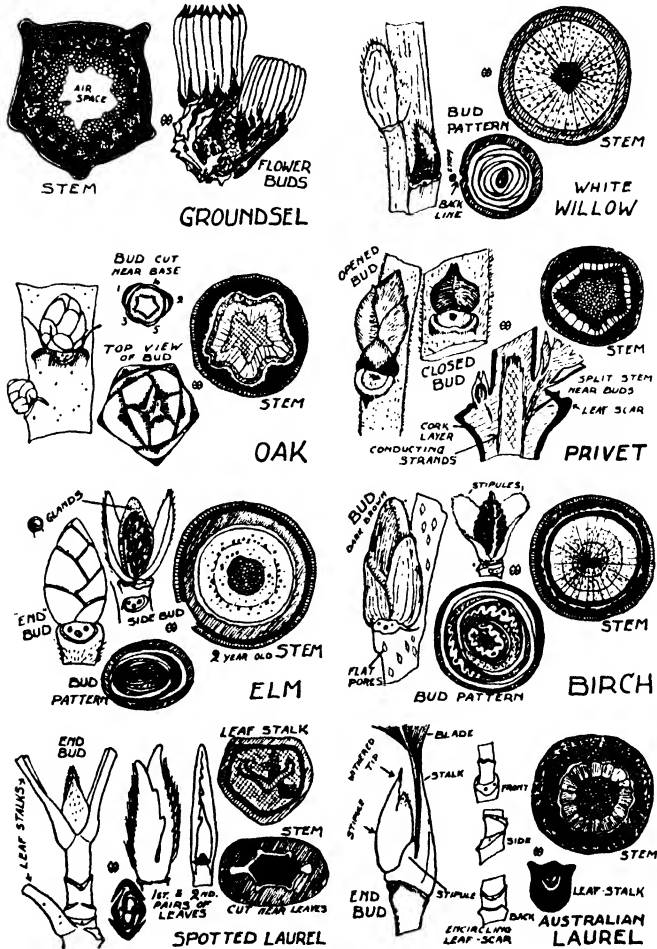


FIG. 20.

pair of very small foliage leaf primordia ; and *Griselinia*, with magnolia-like leaf-base bud-scales.

241. Observe with a pocket-lens, as opportunity arises, the buds and leaf-scars of the following plants :—

With leaf-base scales : rose, cherry-laurel, Portugal laurel, *Aucuba*, sycamore, horse-chestnut, rhododendron, flowering currant.

With leaf-blade scales : lilac, willow, privet, pine.

With entire leaves as outer scales and inner scales : stipules, as in alder, leaf-bases as in ash, or leaf-blades as in holly.

With leaf-stalk scales : elder.

With separate stipular scales : poplar, lime, birch, beech, hornbeam, hazel, oak, hawthorn.

With fused stipular scales : elm, London plane, Spanish chestnut, also *Magnolia* and *Griselinia*.

With old leaf-base protection : *Philadelphus*.

With ordinary foliage leaves folded as protection : carnation, *Saxifraga umbrosa*.

For illustrations see “Pocket-lens Plant Lore.” Small. Churchill.

CHAPTER XIV

BRANCHING AND PHYLLOTAXIS

242. Twigs. (a) Examine twigs of ash showing several years' growth; note that the branching is monopodial. (b) Examine similar twigs of lime or lilac; note that the branching is sympodial. (c) Examine stunted twigs of sycamore; note that the branching may be monopodial and sympodial in alternate years.

Make annotated drawings of all the twigs examined, labelling the buds, leaf-scars, bud-scars and lenticels. Mark in each case the year in which each part of the twig elongated as a new growth from the bud which left the scars at its base.

243. Dichotomy. Examine and make annotated drawings of the prepared specimens of sea-weeds, ferns, and *Philadelphus*. Distinguish between the true dichotomy of the lower plants and the false dichotomy of *Philadelphus*.

244. Adnation. Examine the prepared specimens of *Atropa belladonna*, *Solanum dulcamara*, etc., as examples of adnation. Make a diagram of two specimens to show the relation between the adnate organ and the main stem; noting that the leaf may be fused with the stem in the nightshade, while it may be the branch stem and main stem which are fused in bitter-sweet.

245. Short Shoots. Examine the fresh and prepared specimens of short shoots. Make annotated drawings of at least two of these, labelling the short shoot and the leaf which subtends the latter, e.g. pine, larch, *Mamillaria*, barberry.

PHYLLOTAXIS

246. Leaves. Examine the fresh plants supplied, e.g. groundsel, and plantain or London pride. Determine their approximate phyllotaxis according to the Fibonacci series. Make diagrams to illustrate the $2/5$ and $3/8$ divergences.

247. Determine the approximate phyllotaxis of the other plants supplied, e.g. carnation or other member of the Caryophyllaceæ, iris or leek, *Cyperus* or *Carex*. Make diagrams to illustrate the $1/2$ and $1/3$ divergences.

248. Flowers. Examine the prepared specimen of *Helianthus annuus*. This is a capitulum in fruit with sufficient fruits removed to show the two kinds of curves. Check the numbers of spirals in the curve system.

249. Obtain a young open flower-head of the daisy or other member of the Compositæ, and determine the curve system in your specimen. Compare the smaller number of larger spirals with the number of ray florets in the same specimen. In most cases with one row of ray florets these numbers will be approximately equal, but with "doubling" as in the normal daisy the number of ray florets may be twice that of the more numerous shorter spirals; 42, with a few extra to give about 45; for an illustration see "Pocket-lens Plant Lore," Pl. VII, No. 51.

250. Cone Scales. Examine carefully the cones of (a) a small pine such as *Pinus sylvestris* and (b) a larger type such as *Pinus pinea*. Determine the curve system of each one, and note that the smaller cone may show numbers which are equal and not in the Fibonacci series.

251. Emergences. Examine a fruit of *Raphia* and note that the scales are not foliar in nature but emergences. Determine the curve system. Compare the results of Exs. 246-251 and note that, whether the organs be leaves, flower-buds, cone-scales or merely emergences, they are arranged definitely, as the result of the normal origin of one lateral organ at a time together with a uniform growth rate; and that where two organs arise simultaneously as in Caryophyllaceæ, *Raphia* fruits and some pine cones, two genetical spirals may be traced giving a curve system of equal numbers such as $(6 + 6)$ or $(2 \times 3 + 2 \times 3)$. These bijugate systems may have numbers which are double those of the Fibonacci series.

Fingers

252. Measure carefully on your own left hand the lengths of the three phalanges and of the metacarpal bone of each digit. These should approximate to the Phi ratio series 1.0, 1.618, 2.618, 4.236. As an example of a normal long digit we may take in centimetres 2.5, 4.0, 6.5, 10.5; a shorter example with a short metacarpal bone might be 2.1, 3.4, 5.5, 8.5 cm. Note that the Phi ratio gives an additive series, like the Fibonacci series; and that the Phi ratio, very approximately 5 : 8 or 8 : 13, is a more accurate measure of good proportion than the lower members of the Fibonacci series.

CHAPTER XV

GROWTH

253. Examine a prepared longitudinal section of a **root-tip**. Using a micrometer eyepiece measure carefully the length of every second or every third cell in one of the rows from the apical meristem upwards until you have ten measurements. Plot these on squared paper, using the series 02468, etc., or 0369, etc., as abscissæ. Write a brief explanation of the form of the curve obtained.

Distribution of Growth

254. Root. Choose a germinating broad bean with a straight root about 5 to 8 cm. long (see Ex. 261). Mark the root from tip to top with equidistant lines about 1.5 to 2.0 mm. apart. This may be done with a stretched thread soaked in ink, but a more rapid and convenient method consists of running a wheel with equidistant *rubber* teeth along the root. This graduated wheel or opisometer should be inked with printer's ink or with waterproof Indian ink spread on a glass plate with the edge of a piece of paper. Measure the spaces from mark to mark with a pocket-lens micrometer; fix the marked seedling in a damp chamber, e.g. a beaker lined with blotting paper and one-third full of water, with a piece of sheet cork to receive the seedling.

After one or two days note the altered spacing of the marks, measure the spaces again and record your results as a diagram drawn to scale three times natural size. Note that your results give evidence for a "grand period of growth" for each part of the root, the maximum occurring a few millimetres from the extreme tip (Fig. T 390; see Ex. 267).

255. Flower-stalk. Using either printer's ink or waterproof Indian ink and the opisometer type of rubber wheel, mark a straight flower-stalk with equidistant lines and after one or two days record your results in a diagram as before. Suitable materials are: young budding scapes of *Primula*

or Polyanthus either attached to the plant or cut carefully and kept in a test-tube with a little water; also young dandelion scapes in bud or older scapes with the flower-head closed and the fruits nearly mature.

256. Stem. Mark, in the above manner, the stem of a healthy young plant, and after one or two days record your results in a diagram as before. Suitable materials are young stems of potted plants, e.g. bean, *Phaseolus*, valerian, either potted or growing outdoors. If a twining stem be used curious distortions of the lines may be observed, together with a maximum growth in internodes which were too young to be marked (Fig. T 391).

257. Leaf-stalk. Choose a reasonably straight leaf-stalk of *Tropaeolum*; mark it in the above manner, and after three or four days record your results in a diagram drawn to scale as before (Fig. T 392).

258. Leaf-blade. (a) Using the opisometer wheel as before, mark the so-called leaf-blade of a young daffodil or hyacinth from base to apex and make a diagrammatic record of the result after three or four days.

(b) Using a finely pointed brush and waterproof Indian ink mark two series of equidistant parallel lines, one series at right angles to the other, on the upper surface of a young leaf-blade, left attached to the plant. Draw the outline of the leaf and a careful plan of the marks made. After two to four or more days make another drawing to show the distortion which results from growth in area. Suitable materials are:—young leaves of *Fagopyrum* (Fig. T 393), *Bryophyllum calycinum*, *Helianthus annuus*, *Griselinia*, lilac, cherry-laurel, etc.

Temperature Effects

259. Seedling Stems. Take four $2\frac{1}{2}$ or 3 inch Petri half-capsules, fit them with discs of blotting-paper, three layers of paper in each. Thoroughly wet the paper and sprinkle evenly over the wet surface a supply of small seeds, e.g. cress, mustard, flax, turnip; cover with a bell jar and leave until germinated. If there is any obvious difference amongst the four sets all precocious seedlings should be removed. When four uniform sets A, B, C, D have been obtained, place each one under a bell jar with equal illumination, A near the hot-pipe vent of a greenhouse, B in the normal heat of a greenhouse, C in a cooler glass-house and D over an ice and water mixture. Take the temperatures

of each location daily about an hour after mid-day. Note, compare and take records of the average stem height, general vigour and depth of green. Cress seedlings respond readily to differences in temperature.¹

260. Seedling Roots. Soak about fifty peas in water overnight, pour off most of the water and leave the soaked seeds in the vessel with the top covered. When germination has begun, spread the seeds out in a shallow dish with a little water, and choose carefully four lots of eight, all of which have the radicles just emerging from the seed-coat. Place each set of eight in a closed Petri capsule with wet blotting-paper in the bottom, and arrange the sets as A, B, C, D in the same locations as Ex. 259.

Take the temperature daily and after two days measure carefully all the radicles in each set. Compare the average lengths for the four sets with the temperature differences as recorded.¹

261. Root. The following experiment requires straight, clean, well-grown roots on seedlings of *Vicia faba*, the broad bean.

Germination of Beans. Seedlings with straight clean roots are easily obtained by impaling moistened seeds on ordinary pins and fixing them on a vertical cork-sheet surrounded by a frame, with a screen to keep the light out and the moisture in, and a continuous flow of tap-water over the surface of the cork (see Fig. 21). Broad beans should have two pins,² one to fix the seed firmly to the cork and the second to keep it from being turned after the radicle has grown.

Sachs' Curvature. Broad beans grown in this way may show a curvature in the plane of the cotyledons. This autonomous curvature is known as Sachs' Curvature and such seedlings should be rejected.

Choose a seedling with a suitably straight root about 2 cm. long; arrange this firmly in a museum jar or other vessel with flat glass sides. Sufficient water should be present to immerse the radicle, but leave the cotyledons above the water-surface. The bean root breathes largely by means of an aerating system connected with the other parts of the seedling.³

Using the horizontal microscope with an eye piece micrometer,

¹ Students should note that for precise experiments the temperature should be either controlled and constant or recorded continuously by a Thermograph (Fig. 9).

² The purple-black marks around the pins are probably due to the amino-acid dihydroxyphenylalanine which occurs in all parts of the broad bean and readily oxidises when the tissues are killed.

³ See C. Hunter in "Annals of Botany," 29, 627, 1915.

focus carefully upon the extreme tip of the root-cap. Record the micrometer readings at intervals of five minutes, until the growth rate is constant. Take the temperature of the water and adjust the microscope. Siphon off all the water and add water at a temperature about 10°C . above that of the original until the initial level is again obtained. Note the temperature and the micrometer reading at intervals of five minutes. The rate of

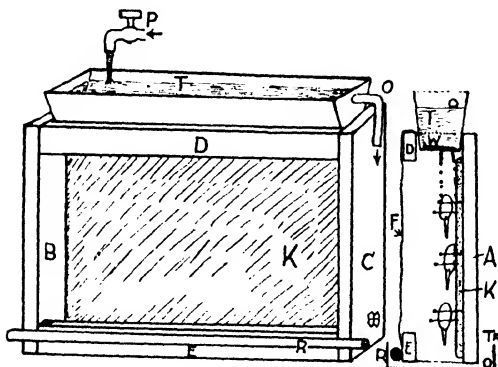


FIG. 21.—*Germinator for straight roots.* Teak, $\frac{3}{4}$ inch thick, and 4 inches wide is used for the sides, BC; connected by $1\frac{1}{2}$ -inch bars, DE. The $\frac{3}{4}$ -inch cork matting K forms the back A; a waterproof screen F is attached to D, and to the roller R. Water from the tap P keeps the trough T full to the overflow pipe O, and drips through the perforated bottom of the tank, the perforations of which have wick-like threads, W, to reduce the flow. All screws are brass. The metal tray Tr with overflow pipe allows of the germinator being set on the bench *beside, not in*, the sink. Water is then supplied to the tank by a rubber connection with the tap.

growth shows an initial increase with rise in temperature, but decreases again as the temperature gradually falls.

When the temperature and growth are again normal and the microscope has been adjusted, siphon off the water as before and replace it by water in which ice has been melted to give a temperature of about 2 to 4°C . Record the temperature and the micrometer readings as before; note the marked decrease in the growth rate.

N.B. Broad bean roots are chosen for this experiment because they are relatively insensitive to light. See also Exs. 264 and 267 (b).

262. Stems. Using an auxanometer to measure the growth

rate, with a thermometer near the plant, and either an electric hot-plate or a 60-watt lamp, covered with black cloth on a frame, to maintain a rise in temperature of the surrounding air, determine the rate of growth in the stem of a potted plant, e.g. bean, sunflower, at normal room temperature and at the higher temperature obtained as above-mentioned. Here, as in the other three cases, increase of temperature in northern laboratories gives an increase in growth rate. In dull light the graph of growth rate in *Vicia faba* stem may be found to follow closely the temperature graph of a Baro-thermograph (Fig. 9).

Oxygen and Respiration

263. Repeat Ex. 254, using two sets, each of four bean seedlings, and filling one jar with water so that cotyledons as well as roots are immersed, while the second set of seedlings are grown in damp air. Remeasure after one or two days, and note that the total root growth of the completely immersed seedlings is only about one-fifth normal.

264. Set up the seedling, horizontal microscope, etc., as in Ex. 261. Determine the normal growth rate with only the radicle immersed up to the top. Add sufficient water to cover the cotyledons completely and record the decrease in the growth rate. Siphon off the excess of water and note the recovery to normal.

265. **Light and Growth Rate.** Choose ten mustard seedlings, mark each radicle with waterproof Indian ink at one point about 1 cm. from the extreme tip of the root. Support the marked seedlings on perforated zinc or by cotton-wool plugs in perforated sheet-cork, in a beaker so that the roots dip into water. Measure each root carefully from mark to tip by means of the horizontal microscope, using the screw adjustment and vernier. A pocket-lens micrometer giving readings within 0.05 mm. is also suitable, and more convenient if the mark be made rather nearer to the root-tip. Fix the beaker on a vertical Klinostat¹ in a well-illuminated position.

Rotate the seedlings for three or four hours, and remeasure.

Cover the beaker with a black cloth and again rotate the seedlings for three or four hours. Remeasure again and note the greater growth rate in the dark as compared with that in the light.

N.B. Mustard seedling roots are chosen for this experiment because they are sensitive to light, but rotation in the light then becomes necessary in order to avoid the curvatures resulting from unequal illumination.

266. Light and Etiolation. The effect of light on the

¹ If Small's Klinostat (Fig. 31) is used a cork plate with a central hole should be placed over the boss and the beaker should be tied to two of the three parallel bars.

formation of chlorophyll is examined in Exs. 173–174. The pale colour is, however, only one of the effects of the absence of ordinary illumination. The student should combine the observations made in Ex. 173 with records of the actual average lengths of the seedlings and shoots grown in light and in darkness. Notes should also be made of the relative development of the leaves under the various light conditions. Sections should be made of the thicker stems in the first, second and third internodes of the leafy shoots; and the relative starch content together with the development of the endodermis noted. See Barton Wright in "Recent Advances in Plant Physiology," pp. 288–289.

Growth and Turgidity

267. Plasmolytic Shrinkage. (a) Ex. 254 may be extended by placing the root of the seedling in a 10 per cent. solution of sodium chloride or potassium nitrate for fifteen to thirty minutes and remeasuring. It should be noted that the distribution of shrinkage in this case is not strictly parallel to the distribution of growth. In other cases these may be almost the same.

(b) Set up Ex. 264, immersing the radicle only, first in water and then in 1 per cent. solution of sodium chloride or potassium nitrate. Note the decrease of growth rate in the salt solution, which reduces turgidity although it may not plasmolyse.

268. Differential Turgidity.

(a) Split one end of a stem into four, longitudinally. Observe the outward bends. Immerse the cut ends in water and note that the curvature increases. Suitable materials are numerous, and include sunflower hypocotyl, bean stem, dandelion flower stalk, also most succulent scapiform flower-stalks and other soft stems.

(b) Take about 25 cm. of rhubarb leaf-stalk, measure it carefully. Peel off *all* the outer skin, being careful to have two or three complete strips which run the whole length. As soon as possible measure the length of the outer strips, which will be shorter, and also the length of the inner part, which will be longer. Use this result in a written interpretation of the results obtained in (a) above.

(c) Split the apical portion of a young plump bean-root into two or three longitudinally. There is no outward curvature. Immerse the split part in water and leave it for half an hour. Any curvature which occurs is inwards.

The orientation of the differential turgidity of the tissues is reversed, the outer being the more turgid.

(d) Treat the pulvinus of *Phaseolus* in the same way. Again the curvature *in water* is inwards, indicating a greater extensibility or a higher suction pressure in the outer tissue.

Auxanometers

Many different patterns of these instruments are available. The following references may prove useful to advanced students and teachers who may desire to improvise their own apparatus:

Older Types: "Textbook of Botany," by Sachs, pp. 746, 747. "Physiology of Plants," by Darwin and Acton, pp. 149 *sqq.*

Pulley, Recording Type: Haines in *Annals of Botany*, 33, 181, 1919.

Electrical Condenser Type: Dowling, in *Phil. Mag.*, 46, p. 81, 1923; also *Nature*, 2, 6, 23.

Optical Lever Types: Bose, "Plant Response," pp. 7, 16, 416, 471. Longmans, 1906.

For Root Growth: Neilson-Jones, *Annals of Botany*, 34, p. 555, 1920. See Ex. 270.

The balanced lever or balanced pulley types give sufficient magnification for the experiment described below. A modification of Dowling's Ultramicrometer has been successfully used for still unpublished investigations in the present writer's department.

269. (a) Using the best available self-recording auxanometer obtain a record of two successive periods of twenty-four hours and plot a graph to show the diurnal periodicity of growth in the stem of a potted plant. Suitable materials

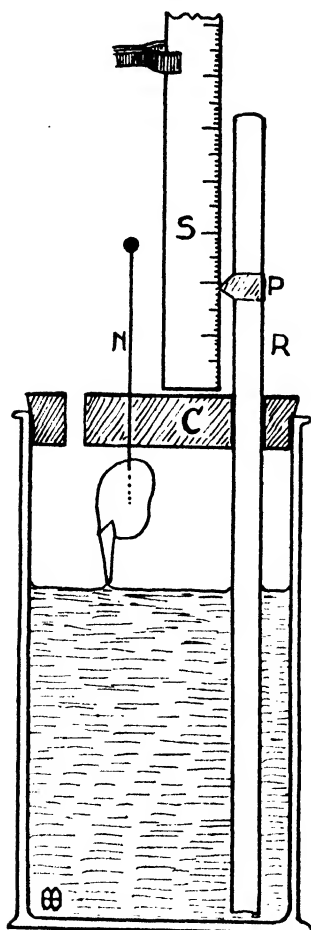


FIG. 22.—Root Auxanometer, for explanation see text (modified after Neilson-Jones).

of twenty-four hours and plot a graph to show the diurnal periodicity of growth in the stem of a potted plant. Suitable materials

are : Narcissus bulbs with young flowering stems, Jerusalem artichokes with young vegetative shoots, and young plants of *Phaseolus*.

(b) Extend the above experiment to include a record of growth variations when the plant is kept in the dark for at least two successive periods of twenty-four hours. The survival of the diurnal periodicity in continuous darkness varies with the plant used.

N.B. A continuous record of the temperature and relative humidity during the experiment, obtained from the Baro-Thermo-Hygrograph (Fig. 9), should be considered in relation to the graphed results. Growth rate may follow the light conditions, or the temperature conditions, or both (see Fig. T 389).

270. The Neilson-Jones Root Auxanometer. Select a cylindrical glass jar of even width, from 5 to 10 cm., also a good cork to fit and a piece of glass rod from 5 to 10 mm., according to the diameter of the jar. Measure the internal diameter of the jar¹ and the diameter of the rod. Bore three holes, two for aeration, through the cork. Fit the rod R (Fig. 22) through the cork C, so that the rod moves easily in its hole. Push a long pin N through the cork ; fix a stiff paper pointer P about 2 cm. long on the rod R. Half fill the jar with water ; transfix a healthy seedling with a straight root upon the pin ; and place the cork firmly in the jar, with the root near the water and the glass rod near the bottom. Fix a centimetre scale S vertically just over the cork by means of a clamp-stand, so that the pointer P will travel along the millimetre divisions when the rod is raised (see Fig. 22).

Manipulate the pin so that the root-tip just touches the water (Fig. 22) ; read the level of the pointer. Withdraw the rod, so that the water-level falls. After five minutes, push the rod down until the water just touches the root ; read the level of the pointer again. Repeat the process, taking as a measure of growth either the time taken for centimetre rises of the rod and pointer or the distance moved by the critical position of the pointer in regular periods of time, e.g. five or ten minutes.

Since this method depends upon displacement over the larger surface of the water by the smaller rod, the magnification varies as the square of the respective diameters (cross-sectional areas) ; thus if the ratio of the two diameters, jar and rod, is 10 : 1, the magnification obtained will be 100, and therefore each centimetre on the scale corresponds to 0.1 mm., each millimetre to 10 microns.

In experiments lasting for more than a few hours, aeration and evaporation require further refinements, for which the original note, *A.B.*, 34, 555, 1920, should be consulted. The addition of the centimetre scale has been made by the present writer in order to facilitate measurement.

¹ This may be done by fitting a strip of stiff paper accurately into the jar and measuring the width of the strip.

CHAPTER XVI

SECONDARY GROWTH

Stems of Dicotyledons

271. Sunflower

Cut transverse sections of an old stem of sunflower (*Helianthus*). Stain in aniline sulphate and mount in dilute glycerine. Make a line diagram showing the position of *interfascicular* and *intrafascicular cambium*, *primary* and *secondary xylem*, and *phloem*. Note the difference in structure of primary and secondary xylem. The primary xylem and primary phloem have been separated by the secondary tissues formed by the cambium.

The sunflower, being an annual, does not show successive annual rings in the secondary xylem. See Figs. T 395 and T 396.

272. Lime, T.S.

Cut transverse sections of the stem of lime tree (*Tilia*). Make a low power line diagram to scale showing : (a) *Epidermis*, possibly absent ; (b) *Cortex* with cork formed from meristem (*phellogen*) towards the outside ; (c) *Stele* containing : (1) fairly wide ring of *phloem* ; (2) *cambium* ; (3) *xylem*, primary and secondary ; (4) *medullary rays*.

The secondary xylem now occupies the greater portion of the stem. *Annual rings* of growth may be seen. The groups of primary xylem project into the pith. *Medullary rays* are seen in T.S. as narrow lines of cells passing from the pith through the xylem and widening out into fan-shaped portions which interrupt the ring of phloem. The cells of the rays have non-lignified walls and often contain starch grains. Under H.P. note and *draw carefully* : (a) *cork cambium*, and *cork* consisting of regularly arranged rectangular cells formed by the phellogen ; (b) *phloem* consisting of alternating bands of : (1) *sieve-tubes*, *companion cells*, *phloem parenchyma* ; and (2) thick-walled *bast fibres* ; (c) *secondary xylem*, consisting of *vessels*, *xylem parenchyma* and *xylem fibres*. A careful H.P. drawing of (c) should include the

junction between autumn and spring wood (cp. Figs. T 397 and T 398).

273. Lime, L.S.

Cut radial longitudinal sections of lime stem (three to four years old). Stain with aniline sulphate and mount in glycerine.

Note and draw the following tissues: (1) band of *cork* which presents the same appearance as in T.S.; (2) *cortical tissue*; (3) *phloem*, the *phloem fibres* appear as narrow elongated pointed cells with very thick walls; in the spaces between the strands of fibres, *sieve-tubes* with oblique terminal walls, each bearing three to five *sieve-plates*, may be seen (look also for companion-cells and *phloem parenchyma*): (4) *cambium* appears as a narrow band of cells with thin walls and abundant protoplasm: (5) *xylem*. Observe the following, all of which have lignified walls: (a) the larger *vessels* with septa partially or completely absorbed; (b) smaller *tracheids* in which no perforation occurs; (c) *xylem fibres*—elongated pointed elements with simple pits on their walls; (d) *xylem parenchyma*—the walls are lignified and pitted, and the cells have living contents, often also starch grains.

The conducting elements of the secondary xylem have both pitted and spiral markings on the same wall.

Examine and draw the medullary rays in the xylem and in the phloem.

274. Timbers

(a) Examine demonstration specimens of oak and other timbers showing secondary thickening; note particularly the annual rings of xylem and the medullary rays.

(b) Examine and note the characters of the woods used in medicine, e.g. sappan, logwood, quassia, guaiacum, berberis, red and yellow sandalwood.

275. Pocket-lens Observations. Examine a series of tree-twigs in transverse slices, or cut surfaces, with a pocket-lens. Make diagrammatic records of your observations, including the annual rings, relative development of large vessels and medullary rays. See "Pocket-lens Plant Lore."

276. Examine prepared sections of stems showing unusual secondary thickening. Make annotated diagrams of the general anatomy and take notes of the points which are departures from the usual arrangement. Suitable materials are transverse

sections of old stems of *Cucurbita*, *Aristolochia* (Fig. T 399), *Clematis* (Fig. T 400), *Dracaena* (Figs. T 402–403), also old woody stems of *Senecio angulatus*, and *Piper* spp.

277. Note the prepared specimens or sections of woody lianes showing abnormal secondary growth, e.g. *Bignonia venusta* and other species, *Serjania* spp., *Bauhinia* spp.

Stems of Gymnosperms

278. Cut and mount a transverse section of a three-year-old twig of *Pinus*. Make an annotated diagram of the general anatomy, and a detailed drawing under H.P. of a small part of the younger secondary wood, to show the regular radial arrangement, bordered pits, and one resin canal (Figs. T 404–405).

279. Examine and draw prepared sections of the old stems of *Ephedra sinica* and *Ephedra equisetina*. Note the absence of large vessels from the former and their presence in the latter section; note also the distribution of the groups of sclerenchymatous fibres in the two sections. *E. sinica* is used in medicine.

Roots

280. **Bean.** Make comparative diagrams of the prepared transverse sections of young and old bean roots. Explain the small size of the old root as compared with its increased xylem and as compared with the thicker younger roots.

281. **Lime.** Cut a transverse section of old root of lime (*Tilia*). Stain in aniline sulphate and mount in glycerine. Make a L.P. line diagram of the whole and a H.P. drawing of part of the section. Note: (a) *centripetal primary xylem*, consisting of *protoxylem* and *metaxylem*, occupying the central part of the root and surrounding the pith; (b) *centrifugal secondary xylem*, surrounding and lying between the groups of primary xylem; (c) *cambium cells* lying between and giving rise to secondary xylem on the inner side and secondary phloem on the outer side; (d) *cork layer*, *phellogen* arises in the pericycle of the root and not in the cortex as in the stem. Remains of dead cortex may possibly be seen outside the cork. Compare Fig. T 407.

282. Cut a transverse section of the thin lower part of a beetroot or other member of the *Chenopodiaceæ*. Make an annotated diagram of the general anatomy and write explanatory notes on the anomalous secondary growth.

283. Cut a series of thin sections of the carrot, from the thin end upwards. Prepare a set of diagrams to show the

arrangement and position of lignified xylem elements, the development of central parenchyma, the position and arrangement of the densely coloured tissues in the phloem zone. Write a brief comparative note on the two ends of the series, including an explanation of the differences.

284. Roots used in Medicine. Examine and sketch the smoothly cut surface of the following medicinal roots or rhizomes, and write explanatory notes on the structures which are seen, using a pocket-lens for details. With abnormal secondary growth or other unusual features: black snakeroot, pareira root, calumba root, Mexican scammony, rhubarb, sumbul, jalap, turpeth (very large vessels), inula root (oil glands), bloodroot (red secretory cells), dandelion (zones of laticiferous tissue). With a more usual structure and variations: liquorice, gelsemium, hellebore, aconite, senega, rhatany, ginger, orris, marshmallow, ipecacuanha, pellitory, gentian, horseradish, belladonna.

Periderm and Barks

285. Phellogen. Cut very thin transverse sections of the outer part of the mature stem of elder (*Sambucus nigra*). Examine these carefully and select a good part of one section; draw this under H.P. to show the form, position and arrangement of cork cells, phellogen and phelloderm.

286. Lenticels. (a) Using the same material as above, cut a thin transverse section passing through a lenticel; draw this to show the incurving phellogen, the closing layer of cork, which may be broken; the loose rounded external complementary cells, and the internal phelloderm. Label all the tissues.

(b) From a sliver of the old woody stem of the elder, cut a comparatively thick section of an old lenticel. Make an annotated diagram to show the alternate broken layers of closing cork cells and the remains of the alternating layers of complementary cells (see "Pocket-lens Plant Lore," Pl. 22, No. 170, for illustration).

287. Cork. Investigate with a pocket-lens the annual zones, lenticels, and cork cells of ordinary bottle cork in both long corks and flat corks (see "P-l. P. L.," No. 151).

288. Origin of Phellogen. Observe, in transverse section under L.P., the relative survival of primary cortex in old branches of *Solanum dulcamara*, barberry, *Ribes* and *Spiraea*, also twigs of sycamore or beech. Pharmaceutical students should also compare with these a transverse section of valerian root.

289. Barks. The form of the phellogen, primary and persistent as in beech and sycamore, or secondary, scaly and curving outwards in various ways according to the large size of the interrupted areas of phellogen as in London plane, or the smaller flat areas as in oak or deeply curved areas as in alder, or very numerous flat areas as in birch; all these and others may be studied either with the unaided eye or with a lens by cutting out a longitudinal strip about 1 cm. wide and 3 cm. long from the boles of the trees and then making smooth cuts across the transverse surface (see "P-l. P. L.," Nos. 152-154, for representative examples).

290. Barks used in Medicine. The secondary outer tissues of many plants contain alkaloids and other medicinal substances. The student should have some acquaintance with examples of these useful barks, *e.g.* relatively smooth with transverse lenticel cracks—wild cherry, alder, buckthorn, cascara sagrada; relatively rough with deep transverse cracks—cinchona, yohimbe, oliver's bark, witch-hazel, cascarilla; relatively smooth—canella, euonymus, cassia; inner barks—cinnamon, slippery elm, quillaia.

Leaf-fall

291. In the autumn, observe the ease with which leaves of deciduous trees may be removed from the twigs, leaving a clean, smooth surface as the beginning of a leaf-scar. Select twigs from two such species and cut a series of longitudinal slices including leaf-base and twig-stem. Observe and sketch the general appearance, and mark carefully the position of the transverse abscission zone. Suitable materials are horse-chestnut, sycamore, alder and lime (see "Pocket-lens Plant Lore," Nos. 149, 150, 156).

292. Extend the previous exercise to the making of permanent preparations, using selected thin longitudinal sections. Examine these under H.P.; draw the details of phellogen, cork, and the abscission phellogen of loose cells between the cork layer of the leaf-scar and the deciduous part of the leaf-base. Suitable materials are twigs of the above-mentioned trees, collected in the autumn and used either fresh or pickled.

Note.—Advanced students and those preparing for pharmacognosy will find "An Introduction to Plant Anatomy," by A. J. Eames and L. H. MacDaniels, very useful.

CHAPTER XVII

CLIMBING PLANTS

Twiners

293. EXAMINE and sketch the specimens of twining stems supplied. Note the direction of the nearer parts of the spiral, rising from left to right or right to left. Draw diagrams of the twiners and their supports as seen from above, each with a curved arrow to indicate the anti-clockwise or clockwise direction of the twining. Suitable materials are pieces of thin supports with the corresponding part of the twining stem of runner bean, great bindweed, black bindweed, hop, honeysuckle and black bryony.

294. Circumnutation. Using potted plants, or if necessary woody shoots recently cut, firmly planted and supported by a cane in moist soil, observe and make a record of the movement of the apex of a twining stem, *e.g.* runner bean, great bindweed, hop, or honeysuckle. A convenient method of obtaining a record is the following: Arrange the pot on the floor; fix a glass plate in a clamp-stand about 3 inches above the stem apex, also a piece of thick cardboard, about 6 inches square, 9 inches above the glass plate. View the stem apex through a small hole in the centre of the cardboard, and mark a dot with Indian ink on the plate, so that the dot, the hole and the stem apex are in alignment. Repeat this every ten or fifteen minutes for two or three hours, numbering the dots in the order in which they are made. Copy the record from the glass plate into your notebook and state the time taken for one circumnutatory circle.

295. Examine and sketch the prepared specimen of *Cuscuta*. Note the points of attachment to the host plant and the direction of the twining, anti-clockwise (Fig. T 297).

Tendrils

296. Examine fresh or prepared specimens of the various kinds of tendrils. Make a careful drawing of each to show the morphological nature of the tendril by means of its

relation to other parts of the plant. Suitable materials are fresh specimens, or if necessary museum specimens, of the following plants with tendrils: pea, vetch, Virginia creeper, *Clematis*, *Tropæolum (tricolorum or majus not minus)*; also *Bryonia dioica*, *Cobæa scandens*, *Smilax*, *Bauhinia*, some species of *Cucurbita* and *Passiflora*, also *Cucumis sativa*, the cucumber.

297. The following experiments should be attempted only on a hot sunny day, preferably in the open.

(a) Select a tendril which has only a *slight curve*, not a hook, near the tip; hold the lower portion of the leaf or stem so that the apical curve of the tendril is horizontally over the dial of a watch with a "second" hand; stimulate the tendril by gently drawing a match-stick or small twig along the inner surface of the curve; then hold it steadily and note the number of seconds which elapse before any curvature can be detected. The "minute" lines or the more sharply curved "second" line of the watch-dial give a reference curve for the tendril. Provided the primary conditions are observed, suitable plants are *Bryonia dioica*, garden pea, sweet pea and other species of *Lathyrus*. Bryony may respond in less than ten seconds.

(b) If a response be obtained in less than three minutes, the above experiment may be extended by selecting a similar tendril and rubbing gently the outer side of the curve; this may not cause movement. The inner side should then be stimulated as before and the outer side again rubbed gently.

(c) A further experiment may be carried out about the same time. Draw the smoothly polished surface of a fountain-pen case along the curve of a fresh tendril. If the vulcanite surface is clean and free from scratches there is no stimulation. Repeat this using the edge of the pen-nib or a rough twig as before.

(d) The localisation of the sensitive area should be tested by attempted stimulation of other parts of the tendril.

Root Climbers

298. (a) Examine and draw on a large scale a small part of young ivy stem with adventitious climbing rootlets. Compare the form of these rootlets with that of rootlets which have been attached to a support. Draw some of the latter on the same scale to show the flattened form.

(b) The young green spheres and old grey discs of the flower-bud tendrils of Virginia creeper (*Ampelopsis veitchii* not *A. hederacea*) should be drawn beside these roots, as a similar adhesive mechanism.

299. Examine and sketch the climbing roots of *Ficus repens* and other available prepared specimens with adventitious climbing roots, e.g. lianes, *Hoya carnosa*, *Tecoma radicans*, *Marcgravia dubia*, etc.

Hook Climbers

300. Examine and draw carefully, under a dissecting microscope or good pocket-lens, a few of the hooks which occur on *Galium aparine*. Note the swollen spreading base and the curve of the thin apical portion.

301. Make two series of outline drawings of the hooked spines from (a) 12 inches of rose stem and (b) 12 inches of bramble or blackberry stem. Note and draw particularly the extremes in the range of variation which may be observed ; these will be very distinct in the bramble and less different in the rose.

Scramblers

302. As opportunity arises observe and make records of the mode of scrambling shown by *Stellaria holostea*, couch-grass, or other hedgerow scramblers.

More advanced students are advised to consult Darwin's "Climbing Plants," and to note the methods of climbing adopted by other exotic plants in the hothouses of public gardens.

CHAPTER XVIII

RESERVE MATERIALS

THE investigation of the distribution of reserve materials and the identification of such substances as occur are largely matters beyond the scope of a first-year course, but second-year students should have a working knowledge of the simpler tests which may be applied, and of the general results of simple investigations. Some very elementary exercises for first-year students are grouped below.

Elementary Exercises

303. Cut a maize fruit, which has been soaked in water, in half longitudinally. Apply iodine solution to the cut surface; map the distribution of starch as indicated by the blue colour. Using a pocket-lens, examine the outer layers of the endosperm for a brown line. Note the light brown colour of parts of the embryo.

Cut a thin section from the clean surface and draw a few of the starch grains under H.P.

Examine the outer cells of the endosperm and draw a few of them to show the granular protein contents.

304. Using iodine solution on thin sections, investigate the nature of the reserve materials in the cotyledons of bean or pea and lupin, also the rhizome of iris collected in the autumn. Draw the starch and protein grains as seen under H.P.

305. Squeeze a small quantity of the milky fluid from germinating barley on to a slide; add a drop of water and a cover-slip. Examine and draw, under H.P., a few of the corroded starch grains, as one example of a stage in the utilisation of reserves.

306. Examine freshly cut sections of dahlia tubers which have been soaked for several weeks in alcohol. Note the inulin crystal-aggregates of various sizes. Draw representative parts of the section to show that these crystalline masses

may be derived from one cell or many cells. The alcohol kills the cells and the water-soluble inulin may crystallise in the alcohol over several cell walls.

307. Using Soudan red or tincture of alkanet, both of which give a red or pink stain with most fats and fixed oils, investigate under the microscope the nature of the reserve materials in sunflower cotyledons, Brazil nut, and coconut endosperm. Draw a few cells of each and indicate the cell contents.

308. Place three squash preparations, or very thin sections, from small portions of the endosperm of a castor bean, on different slides. Apply to one a drop of Soudan red or alkanet and note the positive result for oil. Apply to a second preparation a drop of iodine solution and a cover-slip, note under H.P. the brown colour of the crystalloid. Apply to the third preparation a drop of dilute hydrochloric acid and a cover-slip, note under H.P. the solution of the globoid. Make a careful drawing, under H.P., of a few cells with their contents from the first or second preparation.

Advanced Exercises

309. Starch. Examine, draw under H.P., and measure with an eyepiece micrometer starch grains from a variety of plants, e.g. potato, wheat, barley, rice, maize, bean, pea, arrowroot, sago, ginger, turmeric.

The position and character of the hilum and striations should be noted carefully where they occur.

310. Draw a diagram to scale of each kind of starch grain used in Ex. 309 as seen by polarised light with crossed prisms.

Rotate the upper prism or analyser, and note the similar movement in the image; this indicates the sphæro-crystalline nature of the starch grain. Compare this phenomenon with the similar case of sphæro-crystal aggregates of calcium oxalate.

311. Prepare a diastatic medium by mixing extract of malt with approximately twice its volume of water. Mount a variety of starch grains as above in drops of this freshly prepared medium and examine them from time to time during a period of about three days. Record the various stages of erosion and disintegration.

312. Amyloid. Cut a thin section of the ripe, dry pericarp of *Tropæolum*, mount it in iodine solution and note the blue colour which results from the amyloid content of the cell walls.

SUGARS

313. Mount a thin cross-slice of onion or squill or other suitable

bulb-scale on a slide with a few drops of freshly mixed Fehling's solution ; add a cover-slip and warm carefully for a few minutes over a small flame. Examine under L.P. and note the red precipitate which indicates reducing sugars, *i.e.* pentoses, hexoses or maltose. If a positive result is not obtained, warm another slice with dilute sulphuric acid for five minutes ; irrigate with dilute alkali ; add Fehling's solution and warm as before. A positive result now indicates the presence of sucrose.

314. Osazones. (a) Test-solution of phenyl-hydrazine hydrochloride is prepared freshly by adding about 0.5 gramme of that substance and 1 gramme of solid sodium acetate to 1 c.c. of strong (33 per cent.) acetic acid. The solution to be tested *in vitro* is added, and the whole mixture heated in a test-tube with a plug of cotton-wool at the top for from five minutes to one hour on a boiling water-bath. On cooling slowly the osazone of the sugar crystallises out in a characteristic fashion and the crystal form under the microscope helps in the identification of the kind of sugar.

Glucose and levulose yield fine golden-yellow needles in fan-shaped bundles after fifteen minutes ;

Maltose yields broad lemon-yellow crystals, single or in clusters, after an hour ;

Mannose yields small stellate clusters of yellow crystals after five minutes ;

Arabinose and xylose yield very slender, long, yellow crystals after an hour ;

Sucrose does not yield an osazone itself, but may hydrolyse and form dextrose and levulose osazones.

(b) For use on fresh plant materials, the phenyl-hydrazine hydrochloride is prepared as a 10 per cent. solution in pure glycerine A, and the sodium acetate as a 10 per cent. solution in pure glycerine B, and both are kept separately in amber drop bottles. The solutions A and B are mixed on the slide ; the sections are mounted in the mixture, a cover-slip is added and the preparation heated in a water-oven for at least twenty-four hours. On cooling the distribution of the osazone crystals approximates to that of the sugars in the section. See Mangham in *New Phytologist*, 10, p. 164, 1911.

315. Pentose and Hexose. (a) Prepare a solution of arabinose, hydrolysing the pentosan "araban" by boiling a little gum acacia for five minutes in 5 c.c. of 10 per cent. HCl ; add 5 c.c. strong hydrochloric acid and a few grains of orcin ; warm gently with a *small* flame. Note the bluish purple colour given by pentoses ; this may darken and change rapidly.

(b) Prepare a 1 per cent. solution of dextrose ; take 5 c.c. of this, add 5 c.c. strong hydrochloric acid and a few grains of orcin ; warm gently. Note the red colour given by hexoses.

(c) Pentoses treated with strong hydrochloric acid as above (b),

give a bright red colour when phloroglucinol is added ; hexoses give no colour. See also Ex. 319.

Ketone and Aldehyde Sugars

316. (a) Add to 5 c.c. of Seliwanoff's solution ¹ a few grains of pure levulose (ketone), boil and note the red colour, which may be followed by a red precipitate ; see also Ex. 317.

(b) Add to 5 c.c. of Seliwanoff's solution a few grains of pure dextrose (aldehyde) ; boil and note that no red colour develops.

OTHER CARBOHYDRATES

317. **Inulin.** (a) Examine sections of suitable material which has been immersed in alcohol for at least three weeks. Note the spheroidal aggregates as in Ex. 306. Suitable materials are the underground parts of *Helianthus tuberosus*, *Cichorium intybus*, *Taraxacum officinale*, collected in the autumn.

(b) Using pure inulin in powder apply :

(i.) Seliwanoff's test, see Ex. 316 (a) —red colour is obtained.

(ii.) Fehling's test, red colour or red precipitate is not obtained (distinction from ketone sugars).

318. **Mannan (Hemi-cellulose).** (a) Cut a thin section of a date stone. Draw a few cells, under H.P., to show the thick walls. Add chlor-zinc-iodine and note that the walls give the blue colour of cellulose.

(b) Boil two or three complete cross-slices of the date stone in 2 c.c. dilute sulphuric acid for ten minutes in a test-tube. Neutralise carefully and divide the solution into two halves ; add one half to the " phenyl hydrazine test solution " and warm, see Ex. 314 ; cool and note the rapid formation of osazone crystals. This rapidity is characteristic of mannose into which the mannan or hemi-cellulose is hydrolysed by the acid. Add the other half to prepared Fehling's solution and boil ; reduction by the mono-saccharide mannose occurs. Other sources of mannan hemi-cellulose are vegetable ivory, coffee beans, onion and clover seeds, but complications occur with all these in the above tests.

319. **Pentosans.** (a) For araban, see Ex. 315 (a).

(b) **Xylan.** Using bran, sawdust, chopped straw or ground shells of coconut or walnut as materials, extract about $\frac{1}{2}$ gramme of material with three successive 10 c.c. portions of strong alcohol to remove sugars ; dry the residue carefully without charring, add 5 c.c. strong hydrochloric acid ; warm gently for a few minutes ; and add a few grains of orcinol.² The bluish-purple of pentose reaction may be further confirmed by adding two drops of strong ferric chloride solution and 2 c.c. amyl alcohol. A green colour in the amyl alcohol layer is a further indication of the pentose sugar xylose.

¹ See Appendix II.

² With sawdust and other lignified material a red colour may develop with the acid alone, before orcinol is added ; this is due to the phloroglucinol component of the lignin.

Gums and Mucilages

320. Using separate small quantities of mucilage of acacia (gum acacia mixed with an equal part of water) :

(a) Add alcohol—white flocculent precipitate.

(b) Add solution of lead subacetate¹—granular yellowish precipitate.

Using small fragments of gum acacia recently added to water :

(c) Add corallin soda¹—the particles stain red.

(d) Add two drops of 1 per cent. methylene blue—the particles stain blue.

(e) Add five drops of chlor-zinc-iodine¹—the particles stain bluish.

321. Using thin slices of the leaves of *Bryophyllum calycinum*, apply the test solutions of (b), (c), (d) and (e) above. Note the positive reaction for mucilage in the cells of the bundle sheath and in some rows of hypodermal cells. These same cells give the "acid colour" with brom-cresol purple, diethyl red, methyl red and brom-cresol green, also the "alkaline colour" with brom-phenol blue, indicating an acid reaction of about pH 4.0 (see Chapter XXV.).

322. Pectin. Press out the juice from a small quantity of gooseberries or red currants, using a plant-press (Fig. 14) ; allow the juice to clear as much as possible by subsidence. Decant the upper portion and add three times its volume of strong alcohol (98 per cent.). Filter off the gelatinous precipitate and dissolve some of it in a small quantity of water. Using 2 c.c. portions of the solution :

(a) Add strong hydrochloric acid—no precipitate.

(b) Add 5 per cent. calcium chloride solution—no precipitate.

(c) Add enough potassium hydroxide solution to render the mixture alkaline ; set aside for fifteen minutes, repeat test (a) as above—the mixture, which now contains pectic acid, gelatinises.

(d) Treat as for (c), but add calcium chloride instead of acid (see (b) above)—a gelatinous precipitate composed of calcium pectate separates.

(e) and (f). Apply the pentose tests given in Exs. 315 and 319 ; pentose reactions are obtained.

PROTEINS

323. Compare in thin sections of the seed, stained with iodine, the protein granules of bean, pea, lupin and cereals with the aleurone grains of *Ricinus*, Brazil nut, sweet almond, pine kernels or arca nut, which have a membrane and a crystalloid and globoid.

324. Using some of the materials as above, Ex. 323, apply to

¹ See Appendix II.

thin sections the following tests, and observe the results under the microscope :

(a) Mount in iodine solution—proteins are stained brown or yellow.

(b) Mount in reasonably fresh Millon's reagent ¹ and set aside for ten to thirty minutes—proteins are stained a brick-red colour.

(c) Mount in 50 per cent. HNO₃ and warm—proteins are stained yellow. Add a few drops of a strong solution of potassium hydroxide—the yellow is intensified.

(d) Mount in a saturated aqueous solution of picric acid ; set aside for ten minutes and transfer to water—proteins are stained yellow.

325. Albumin. Shake 1 gramme of whole wheaten flour with 10 c.c. of water ; set aside, shaking occasionally, for about four hours ; filter ; warm slowly ; albumin is precipitated when the mixture boils.

326. Globulin. Crush three castor beans or one Brazil nut in a small mortar ; add 30 c.c. of 5 per cent. aqueous sodium chloride ; transfer to an evaporating basin on a water-bath ; warm carefully below 60° C. and triturate occasionally for two to four hours ; filter while warm, separate the filtrate into five portions, and apply the following tests.

(a) Cool—globulin may crystallise out.

(b) Boil—globulin may precipitate.

(c) Add a little acid—globulin may precipitate.

(d) Add enough sulphate of ammonia or of sodium to saturate the solution ; if no precipitate is formed, raise the temperature gradually on a water-bath to 32°–35° C.—globulin precipitates.

(e) Transfer the fifth portion to a tube of 1 cm. bore ² with a membrane of gold-beater's skin across one end ; set this aside suspended just under the surface of about 100 c.c. of water in a beaker, for dialysis of the salt. After one or two days remove the membrane and examine its inner surface, under L.P., for crystals of globulin. The results of tests (a) to (e) depend upon the degree of success in the original extraction of the globulin by the salt solution.

Note. The conjugated proteins and derived proteins do not occur as reserve materials to the same extent as albumins and globulins. Advanced students will find the experimental procedure, many other details and further tests for proteins of various kinds in Onslow's " Practical Plant Biochemistry."

FATS AND FIXED OILS

327. Fixed Oils. Using thin sections of the cotyledons of

¹ See Appendix II.

² Such as a straight soda-lime tube or a test-tube with the bottom fused and blown out.

sunflower, the endosperm of castor bean or coconut, or Brazil nut kernel, apply the following tests and examine the results under the microscope :

(a) Mount in tincture of alkanet—oil globules stain red or pink.

(b) Mount in 1 per cent. aqueous solution of osmic acid—oil globules stain brown, becoming gradually almost black.

(c) Mount in ammoniacal solution of potassium hydroxide ; warm gently—fixed oil is saponified.

(d) Set aside in a small stoppered tube of equal parts of alcohol and ether ; mount in the ether-alcohol with a cover-slip—oil globules disappear, passing into solution.

(e) Repeat as for (d), using 90 per cent. alcohol and no ether—all except *Ricinus* show no solution of the oil globules.

328. Fats and Oil Plasm. The usual microchemical tests may be unsatisfactory with solid fats and also with oil which is protected by emulsification in the cytoplasm.

(a) Examine thin sections of cocoa (*Theobroma*) kernels ; note the more or less distinctly radiate structure of the large colourless masses in most of the cells. Mount a section in a few drops of water ; add a cover-slip and warm gently over a small flame, until the water boils ; note that the fat is now in the form of globules which readily give the usual tests (Ex. 327 (a) to (e)).

(b) Examine thin sections of fennel fruit (*Fœniculum*) ; note the granular contents of the endosperm cells ; heat carefully to the boiling point as before and observe the resulting liberation of the oil from the *oil plasm*, as free globules. This globule formation may also be observed by warming gently in chloral hydrate solution.

(c) Cut a few thin sections from a flat cross-cut of the dry seed of *Strychnos nux-vomica*. Flatten one of these as much as possible in the dry state ; mount it under a cover-slip in alcohol ; observe the cell walls near the thinner edge of the section. Irrigate with aqueous iodine, watching the section in the meantime ; observe the extensive swelling of the cell walls ; warm the preparation gently and note the further swelling of the cell walls. These extraordinary phenomena indicate that the cell wall in this case is a mucilaginous cellulose. Note the *plasmodesma* or system of connecting threads of cytoplasm passing through the walls.

Mount another section under a cover-slip in cold water ; observe the granular contents of the endosperm cells, which stain brown with iodine (indicating protein) and do not *readily* give the alkanet or osmic acid tests for fat.

Heat to the boiling point ; note that the *fat* is now freed from the oil plasm in the form of globules which readily give the usual tests as in Ex. 327 (a) to (e).

329. Volatile Oil. This is usually present in small quantities and may be detected by its odour, red stain with alkanet, solubility in 90 per cent. alcohol and absence of saponification with

ammoniacal potash. Globules may be found in sections of orange or lemon peel and in the lower part of clove flower-buds.

GLUCOSIDES

330. Cyanogenetic Glucosides. The enzymic hydrolysis of these, which yields prussic acid, involves crushing the tissues to bring the enzyme into contact with the glucoside, and this process is hastened by killing the cells with chloroform. Three corked test-tubes are fitted with sodium picrate paper¹ between the cork and the top of the tube.

The uninjured material is placed in one tube A, as a control; the crushed material is placed in the other two tubes BC, to one of which, C, a few drops of chloroform are added. The yellow picrate paper remains yellow in A; changes slowly through orange to pink or red in B; and rapidly to pink or red in C.

Suitable materials are cherry-laurel leaves, bitter almonds, apple seeds, also fresh plants of *Lotus corniculatus* and leaves of *Arum maculatum*.

331. Saponins. Add to 50 c.c. of water in a bottle of 500 c.c. capacity, a few scrapings of quillaia bark or Saponaria root. Shake vigorously at intervals during twenty-four hours; frothing indicates saponin. Transfer about 2 c.c. of the liquid to a test-tube; add 1 c.c. dilute sulphuric acid; boil gently for five minutes; neutralise; add fresh Fehling's solution; warm gently; reducing sugars produced by hydrolysis will give reduction.

332. Salicin. Boil a little of this glucoside with 5 c.c. dilute sulphuric acid, and divide into two portions. To one portion add ferric chloride solution, when a purple colour will indicate salicylic alcohol, one of the products of hydrolysis. Neutralise the other portion, evaporate on a water-bath to small bulk; add fresh Fehling's solution, when reduction will indicate glucose, the other product of hydrolysis. The first test may also be obtained by boiling the bark of *Salix alba*, *S. fragilis*, or *Populus tremula* or the flowers of *Ulmaria palustris*, with dilute sulphuric acid for five minutes and adding ferric chloride to the clear supernatant liquid. The red element in the colour distinguishes this from the pure blue or green of the tannin coloration, see below.

333. Tannins. These often occur as glucosides and may be extracted by boiling comminuted or ground material with water and filtering. On the addition of ferric chloride to such a solution a bluish-black indicates pyrogallol tannins and a greenish-black colour indicates catechol tannins. Since other substances also give these colour reactions, precipitation by Braemer's reagent¹ should be used as a confirmatory test.²

¹ See Appendix II.

² See Appendix II, also Onslow for other confirmatory tests.

Suitable materials are powdered oak galls, young bark or leaves of oak and sweet chestnut, which yield pyrogallol tannins ; and bark from the twigs of horse-chestnut, walnut or larch which yield catechol tannins.

334. The following scheme is suitable for second-year students as a qualitative investigation of the reserve substances in a plant or part of a plant as supplied :

(a) Crush some of the material ; add enough water to make a mush and set aside with occasional trituration while the following tests are made.

(b) Cut some thin sections of the material ; mount one section in water and look for starch grains, thick cell walls, aleurone grains, amorphous protein granules, oil globules, fat granules and oil plasm.

(c) Mount a section in each of the following reagents and note the reactions : (i.) iodine solution, (ii.) chlor-zinc-iodine, (iii.) Soudan red or tincture of alkanet. If positive results are obtained for starch, amyloid, mucilage, hemi-cellulose, protein, oil or fat, apply the confirmatory and distinguishing tests given in the preceding exercises.

(d) Take half the mush from (a) together with any free liquid. Shake occasionally with ten times its volume of water for half an hour ; filter ; to successive small quantities of the filtrate apply the following tests :

(i.) Add Fehling's solution and boil—reducing sugars.

(ii.) If the result of (i.) is negative, add dilute sulphuric acid—precipitate indicates globulin or other protein ; boil for five minutes, neutralise and add Fehling's solution—sucrose.

(iii.) Add ferric chloride—tannins.

(iv.) If the result of (iii.) is negative, boil for five minutes with dilute sulphuric acid and add ferric chloride—glucosides of the salicin type.

(v.) Warm slowly to boiling point—albumin or globulin.

(vi.) Add three times the volume of 98 per cent. alcohol—flocculent precipitate is pectin or proteins.

(e) Take half of the remaining mush and digest it with 5 per cent. aqueous sodium chloride for two to four hours ; cool ; if no globulin separates, saturate with sodium sulphate—globulin.

(f) Boil the remainder of the mush with enough water to keep it covered for ten minutes ; filter and apply the tests (d), (iii.) and (iv.) above—tannins and glucosides.

This test need not be used if positive results are obtained with the unboiled aqueous extract.

(g) Test for cyanogenetic glucosides and saponins as above (Exs. 330–331).

More advanced students should work from Onslow's " Practical

Plant Biochemistry," and obtain more than a scrappy idea of the reserve materials of plants. From the point of view of the exploitation of plant reserves as sources of foods and other commercial products the main botany student should be familiar with the facts, experiments and tests given in that volume.

CHAPTER XIX

THE FLOWER

THE flower, both as a seed-producing mechanism and as a conspicuous distinguishing characteristic of the various natural families considered in Chapter XL, requires careful examination. The records taken by the student of the flowers which he examines should be reasonably complete and comparable one with another. The first exercise, therefore, consists of a detailed record of one particular flower as an example of the method to be used later on. The primrose is chosen because these blooms or others of near relatives are available either all the year round in mild situations or for most of the year in temperate regions. The biology of seed production is studied in later chapters.

335. Examine a **primrose flower**; the green outer part ends in five teeth indicating five **sepals** which are fused below to form a loosely fitting tubular calyx. The whole of the outer surface of the calyx and of the flower stalk or **peduncle** is covered with simple clothing hairs. The yellow inner part or corolla consists of a relatively narrow tube, spreading at the top into five lobes, each lobe having a shallow median notch; five **petals** fused are indicated. Attached, by very short stalks or **filaments**, to the inner surface of the corolla are five **stamens** (the andrœcium), which may be situated either half-way down the tube (Fig. 23) or near the top of the tube (Fig. T 459). In the case of the first type it is necessary to split open the corolla tube in order to see and count the stamens, which consist of the yellow plump portion or **anther** and the short stalk or **filament**.

Having split the calyx tube, removing the front half, and having carefully removed the corolla with stamens attached, examine the green innermost part or **gynœcium**. This consists of a more or less spherical seed case or ovary surmounted by a slender **style** bearing a nearly spherical green **stigma**, which is situated at the top of the corolla tube in

the pin-eyed primrose (Fig. 23), but half-way down in the thrum-eyed primrose (Fig. T 459). Remove the rest of the calyx and most of the style with its stigma; using a good pocket-lens, examine carefully the surface of the ovary. Five mounds or valves may be distinguished by looking down on the smooth green surface, but this character is somewhat variable. The presence of five *valves* indicates five **carpels** fused. Note that the calyx and corolla can be easily removed from *below* the ovary, which is therefore superior.

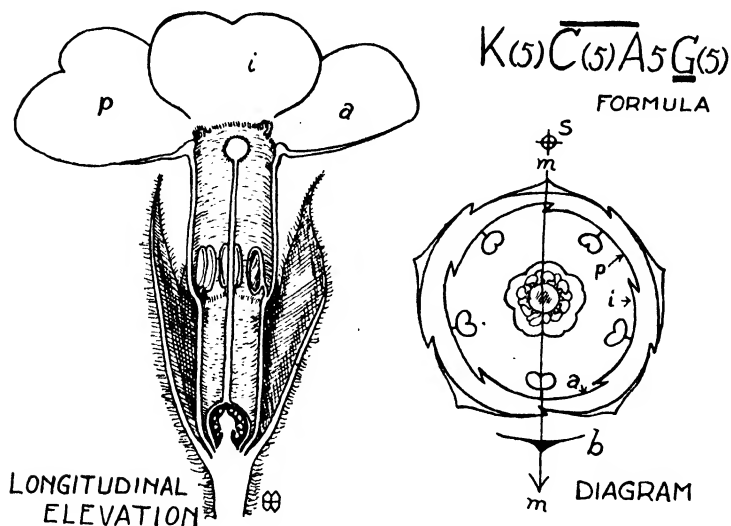


FIG. 23.—Record for primrose flower. Floral formula; floral diagram; and longitudinal elevation; *p*. one of the posterior petals; *i*. inner side petal; *a*. right-half of anterior petal.

Floral Formula. Using K for calyx, C for corolla, A for andrœcium, G for gynœcium, brackets to indicate fusion where it occurs within a whorl of similar parts, a line above connecting the C and A to indicate fusion of members of one whorl with those of another whorl, and finally a line below the G to indicate a superior ovary, we can write the floral formula as a result of the above examination in the form used in Fig. 23, FORMULA.

Floral Diagram. Apart from the fusions, the relation of the members within each whorl, and also the relation of the

members of one whorl to members of other whorls, are not expressed in the floral formula. The floral diagram is, therefore, used to record briefly the methods of overlapping (**æstivation**) and alternation of members of successive whorls. The primrose illustrates alternation of sepals and petals, stamens and carpels, together with the absence of alternation in the succession petals and stamens. **Cohesion** is indicated by lines joining members of the same whorl; **adhesion** is indicated similarly by lines joining the stamens to the petals. This diagram can be orientated, with the main stem indicated above, as at *s.* (Fig. 23), and the bract below as at *b.* (Fig. 23). In the case of the primrose the main stem is very much shortened, but the bract may be found, if the whole plant is available, as the rosette leaf in the axil of which the flower and flower-stalk arise. The overlapping scheme of the sepals may be found in a flower bud, but not in an open bloom; that of the petals is clear even in the older flower. The position of the anthers, one opposite the middle of each petal, is clear in a split corolla. The internal arrangement of the ovary, with a central axis surrounded by ovules and a thin outer wall, can be seen by cutting the ovary across with a sharp razor. The orientation of the valves can be determined by leaving a known part of the calyx attached while examining the ovary. These additional data allow of the floral diagram being constructed as in Fig. 23 **DIAGRAM.**

Longitudinal Elevation. The floral formula and floral diagram contain most of the essential characteristics of the flower, but the general appearance and various details such as shapes, degrees of fusion, insertion of the parts upon the receptacle, nectaries, etc., are better shown by a longitudinal elevation, made as a drawing of one half of a flower which has been carefully split down the median plane, see the line *mm.* (Fig. 23). Either half may be drawn and the floral diagram should be used as a guide in order to get the details correct. All cut surfaces should be represented by double lines, and the drawing should never be *less* than 4 inches long. The illustration Fig. 23 has been reduced for printing. The ovary must be cut longitudinally in half and examined with a pocket-lens. In the primrose the placentation is free central and all longitudinal cuts are similar; but care and reference to the floral diagram are required in cases where the median plane passes through

an ovary partition or septum giving a plain surface instead of a group of ovules (cp. Figs. T 1323 and T 1326).

336. Floral Development. (a) Make line diagrams to illustrate at least four stages in development of the flower, as shown in prepared sections, e.g. *Capsella* series, *Calendula* L.S. young flower head (see Fig. T 440-442, also Fig. 24).

(b) Advanced students should use young flower-buds of

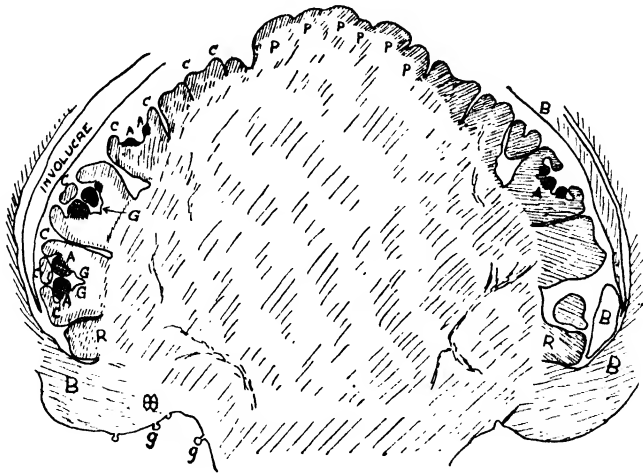


FIG. 24.—*Calendula*—outlines of section through a young flower head; B. involucre bracts; g. glands; R. ray floret with retarded development; CC. primordia of petals; AA. primordia of stamens; GG. early stage in primordium of gynæcium; pp. very young undifferentiated primordia of central disc florets.

Cheiranthus, *Capsella* or *Calendula* upon which to practise the method of serial sections on material embedded in paraffin. The resulting preparations may yield many useful sections for study of the details of floral development.

337. Teratology. The morphological nature of the floral organs may be illustrated by many prepared specimens of abnormalities, i.e. teratological museum specimens of virescence¹ of petals, stamens or carpels, proliferation with the prolongation

¹ *Virescence* is a term used to cover the change of any floral part into a more or less normal green leaf.

of the floral axis, the vegetative development of the axis beyond the lower whorls (Fig. T 450), etc.

Gynœcium

338. Parts. Taking the flowers of (i.) the tulip and (ii.) the daffodil as examples with respectively a superior ovary and an inferior ovary, examine the gynœcium in detail. Note the lower plump part, with three angular rounded swellings giving a more or less triangular seed-case or ovary; the short style of tulip and the longer style of the daffodil which in each case is just long enough to bring the glandular stigmata near the mouth of the flower, where the stamens are located. Draw the gynœcium of each flower on a large scale and label all the external parts. Examine under L.P. and make a still larger drawing of the stigmatic lobes to show the general shape and also the glands of the stigmatic surfaces.

(a) Cut one ovary of the daffodil in half longitudinally; note and draw the arrangement of the ovules in relation to the central axis. Cut another ovary of the daffodil in half transversely; note and draw the trilocular arrangement with ovules on axile placentæ. Examine the cut surface under a lens; note the unusually large embryo sacs in the ovules which have been cut. Draw a few ovules as seen under the lens.

(b) Examine prepared sections, or cut thin transverse sections, of the daffodil ovary. Make a line diagram to show the ovary wall and partitions with the conducting strands and placentæ. Make a labelled or annotated drawing of one ovule, under L.P., to show the funicle, chalaza, integuments, micropyle, nucellus, and embryo sac with antipodal cells, primary endosperm nucleus, synergids and oosphere (Fig. T 520).

339. Megaspores and Development of Ovule

(a) Examine a prepared section showing the tetrad of megaspores in a young ovule, also other stages as available (Figs. T 521, T 522). Compositæ flower-heads yield such sections readily, if buds just beginning to spread the involucre are used.

(b) Examine and draw a series of prepared sections of *Lilium* ovules, showing the unusual transition of the archesporial cell, through the spore-mother-cell stage to the uninucleate embryo sac without division. Continue the series through the meiotic

divisions which here result in the 4-nucleate stage of the embryo sac and by a subsequent mitotic division in the 8-nucleate stage, followed by a 7-nucleate stage when two of the polar nuclei have fused to form the primary endosperm nucleus. Annotate your drawings fully.

340. Types of Ovules. Using the low power of the microscope on young seed-cases or a pocket-lens with dissections of mature seeds, examine and make diagrams to show the relative positions of the funicle or hilum and raphe if present, the chalaza or apex of the embryonic axis if seeds are used, and the micropyle or the root apex in seeds. The seed-cases or seeds should be selected to illustrate the different types of ovules as follows :—

Anatropous—daffodil, castor bean, *Geranium*, *Limnanthes* ;

Atropous or orthotropous—dock, *Polygonum*, walnut, hop, mistletoe (see Ex. 376) ;

Amphitropous or semi-anatropous—primrose, plantain, colchicum ;

Campylotropous—wallflower, broad bean, peas, mallow, hollyhock, *Dianthus*, *Gypsophila*.

Andrœcium

341. Examine the andrœcium of (a) tulip, and (b) wallflower. In each case there are six stamens, each with a stalk or filament and a lobed upper part, the anther. The two short filaments of the wallflower have each a dark green, four-lobed nectary at the base.

Cut thin cross-sections of young unopened anthers from both flowers. The general structure is the same in both the monocotyledon and the dicotyledon. The sections show two large lobes, each of which is two-lobed, showing four pollen sacs in each anther ; in very young anthers there may be a partition. Note the wall of the sac with a small-celled conducting strand in the connective or tissue which joins the two large lobes, and the distribution of the cells with thickened bars on their walls in layers or a layer below the epidermis and in the connective ; also the central masses of pollen grains or microspores, which in younger anthers may be surrounded by a broken layer of dense cells, the tapetum, lying between the pollen and the wall. Observe carefully the smaller cells of the endothecium in the region where the two minor lobes meet ; this is the line of dehiscence.

Make annotated drawings of your preparations.

342. Pollen. Mount pollen grains from mature or nearly mature anthers in glycerol or clear them in chloral hydrate. Examine and draw the grains carefully under H.P. to show the

outer wall with thin areas, germination pores, the inner smooth wall and granular contents including the two or three nuclei if visible. Suitable materials can be obtained from the flowers of tulip, wallflower, iris, foxglove, etc.

343. Examine a series of prepared sections showing the stages in development of the pollen sac or microsporangium. Draw small parts of representative sections under H.P. to show meiosis

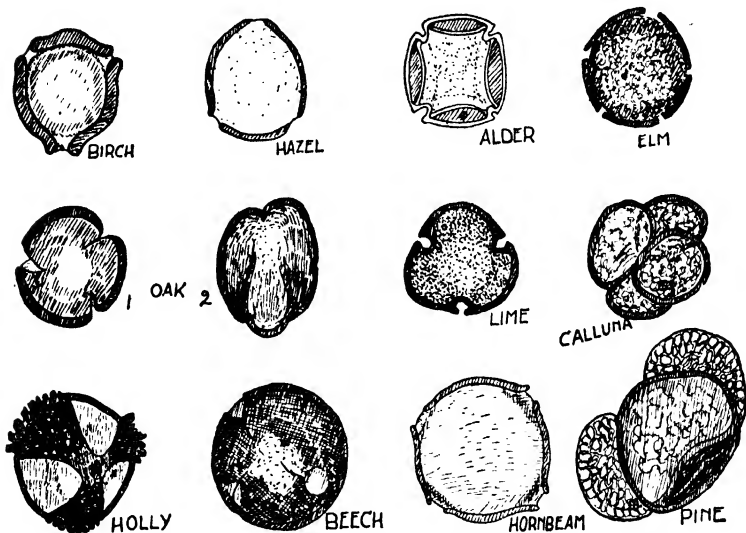


FIG. 25.—*Peat Pollens*. For explanation see text. All except pine and *Calluna*, modified after Erdtman and drawn to the same scale, x500.

and tetrad formation, tapetum and endothecium (Figs. T 572–573).

344. Mount in water, wetting if necessary with alcohol and then irrigating with water, pollen grains from a few of the following plants: sea pink, herb-robert, tree mallow and other mallows with large grains; dandelion, dahlia, sunflower, groundsel, and hollyhock with spines and sculpturing; *Acacia*, *Calluna*, *Erica* and other ericaceous plants with four grains in a persistent tetrad; cucumber, vegetable marrow, white bryony and other Cucurbitaceæ with special pores (Figs. T 565–573). Draw characteristic views of each kind of pollen examined. Note that in the anthers of hybrids and elsewhere the pollen grains may vary considerably in size, e.g. *Oenothera* varieties, *Dianthus* hybrids, *Lavatera*.

N.B. The sculpturing of the exine is often more readily seen after clearing on the slide in a drop of hot chloral hydrate solution.

345. Peat Pollen. The cutinised exine of pollen grains is very resistant to the processes of decay, and as a result the form of these grains, which is characteristic for many species, can still be detected in peat several thousands of years old. The peat (3 grammes) is heated for six hours with 10 c.c. 10 per cent. sodium hydroxide at 110° C. The resulting mixture is diluted to 20 c.c. with water, well shaken, set aside for five minutes. The supernatant fluid is then decanted and a few drops of it examined for pollen grains.¹ The characteristic pollen grains of peat are given in Fig. 25. Lime, elm, holly and hornbeam are sometimes rare or absent; occasionally pine and beech are also absent; the very common types are hazel, birch, alder, oak, together with ericaceous pollen and *Lycopodium* spores.

Perianth

346. Compare carefully the undifferentiated perianth of anemone, marsh marigold, tulip or hyacinth, with the perianth differentiated into calyx and corolla which occurs in lesser celandine, buttercup, wild rose or hawthorn.

347. Polygala. Examine, with a dissecting microscope, all the details of the floral structure in the milkwort, *Polygala vulgaris*. Write the floral formula; draw the floral diagram and a longitudinal elevation; draw also the peculiar details of the perianth, andræcium and gynœcium (Figs. T 731-736).

¹ With suitable refinements, the density and relative proportions of the various tree pollens may be used as indicating the nature of the neighbouring arboreal vegetation at various periods corresponding to the different levels of the peat samples, see *Proc. R.I.A.*, 39, B20, p. 449; also *Journal of Ecology*, 16, p. 317.

CHAPTER XX

THE INFLORESCENCE ¹

MANY inflorescences are characteristic of certain families, in the study of which these examples will be considered. Dried plants in fruit are, however, very useful for a general survey of the methods of growth and grouping in the different types of inflorescences. The examples given below have been chosen so that material may be readily obtained, also usually readily dried and stored for repeated use.

348. Examine and make clear sketches or diagrams of the following inflorescences :—

Single

Scape—daffodil, tulip ;

Scapiform—*Hieracium pilosella*, *Taraxacum*, coltsfoot.

Racemes

Simple, bracteate—snapdragon, foxglove ;

Simple, ebracteate—wallflower ;

Spicate—*Veronica* woody species ;

Spikelet—ryegrass, cocksfoot ;

Spike—plantain (*Plantago* spp.) ;

Catkins—willow, poplar (staminate and carpellate) ;

Strobile—hop ;

Spadix—*Acorus calamus*, or fresh *Arum maculatum* ;

Corymb—units of candytuft (*Iberis amara*) ;

Umbel—*Astrantia* or units of *Heracleum sphondylium* or *Myrrhis odorata* ;

Capitulum—sunflower, or dried young flowering heads of *Calendula* ;

Panicle—oats ;

¹ The elementary study of the contents of this chapter is mainly more suitable for school or nature-study work ; but it is included here in order to preserve the continuity of the exposition and to indicate what practical knowledge the university student should have, as a background for the theoretical consideration of the subject.

- Compound spike—wheat ;
 Compound umbel—hogweed ;
 Compound capitulum—*Echinops*, edelweiss (*Leontopodium alpinum*).

Cymes

- Dichasium—*Erythraea*, *Lychnis dioica* ;
 Polychasium—*Laurustinus*, *Sedum spectabile* ;
 Monochasia—
 Helicoid cyme (bostryx)—terminal parts of St. John Wort ;
 Scorpioid cyme (cincinnus)—middle or basal parts of St. John Wort ;
 Boragoid (coiled cincinnus)—comfrey ;
 Drepanium—sea lavender, *Statice* sp. ;
 Rhipidium—iris, whole inflorescence ;
 Cymose umbel—carrot ;
 Cymose head—sea pink (*Armeria maritima*), young heads of field scabious, fruiting heads of *Platanus* ;
 Cyathium—*Euphorbia*, fig ;
 Verticillaster—*Mentha* spp. *Calamintha*, *Stachys* spp. in fruit, *Lamium album* fresh in bud ;
 Thyrsus—lilac ;
 Reversed raceme—*Nigella*.
 Illustrations of most of the above inflorescences will be found in the Textbook.

CHAPTER XXI

POLLINATION

THE mechanisms for cross-pollination and the natural functioning of these are essentially matters for outdoor study as opportunities arise. The student is advised to take an interest in the natural activities of insects in relation to flowers, especially on days of warm sunshine with little or no wind. Controlled experiment is, however, an essential process in the scientific study of pollination.

349. Using whatever suitable material may be convenient, emasculate four flowers—A, B, C, D—by removing entirely the unopened anthers, using tweezers so that the ovaries remain uninjured. Cover A loosely with muslin tied around the flower stalk; dust over the stigma of B with pollen from another flower of the same species; treat C in the same way with pollen from an unrelated species and “bag” both B and C with muslin as before; leave D open, unprotected by muslin from cross-pollination, and “bag” a fifth flower, E, with the anthers still in place.

Suitable material consists of flowers which are not protogynous and which are large enough to be “bagged” conveniently, *e.g.* foxglove, snapdragon, rose-bay willow herb, wallflower. Compositæ flower-heads are not suitable, for various reasons. After seven or eight days note the effects of the conditions on the development of mature seeds. Write an account of your methods and an explanation of the results.

350. Attempt to prevent seed formation, as in Ex. 349 A, by cutting off with a razor the whole of the upper parts of the corollas in a young flower-head of hawkweed, dandelion or groundsel. Record, and explain as far as you can, the results of this experiment as observed two or three weeks later.

351. Using very young unopened flowers of sweet pea or garden pea, emasculate two blooms, cross-pollinate one, and “bag” both these and a third normal flower. Record and explain the consequent distribution of seed production, after an interval of two weeks.

352. Procure fresh material of *Arum maculatum* in spring. Investigate fully in a young unopened spadix by means of pocket-lens observations and microscopic preparations the following points: colour scheme, nectar glands on spathe, source of colour in spathe and in spadix, modified female flowers as trap mechanism, male flowers, rough-walled carpels, smooth-walled carpels with colourless stigmatic hairs which are flooded with a colourless secretion soon after they are exposed to the air. Compare, in transverse sections, the structure of the purple club, the axis of the spadix, the stalk of the spadix and the stem below the spathe. Make a series of illustrative line diagrams and write an explanation of the observed differences and similarities (see Figs. T 1318-1322, and "Pocket-lens Plant Lore," No. 70).

353. As opportunities arise, note particularly examples of the following structures or mechanisms:

Anemophily

Pine and plantain pollen grains, stigmata of hazel, plantain and grasses.

Nectaries

Petal depressions or pitchers in the series—*Ranunculus acris*, *R. auricomus*, *Helleborus*, *Aconitum*;

Near or on stamens—wallflower, *Arabis*, *Viola* spp.;

On carpel walls—marsh marigold, tulip;

At base of style—sunflower and other Compositae;

Epigynous disc—Umbelliferae, Rutaceae;

Concealed nectar—*Lychnis*, *Gypsophila*, *Epilobium*, *Nicotiana*, *Myosotis*, *Anchusa*, *Symphytum*;

Extra-floral nectaries—cherry-laurel and other species of *Prunus* which all have nectar glands near the base of the leaf blade, *Vicia sepium* (see "P.-l. P. L.," No. 67), *Viburnum opulus*.

Other Mechanisms

Explosive pollen dispersal—nettle, gorse, medick, *Kalmia*;

Swinging anthers—*Salvia*;

Robbery—comfrey, monkshood; Also—

Monœcism—birch, hazel;

Diœcism—dog's mercury;

Protandrous dichogamy—Compositae disc florets;

Protogynous dichogamy—*Plantago* spp.;

Heterostyly—*Primula* spp., *Lythrum* spp.;

Cleistogamy—autumn dog violet, sundew;

Polygamy—ash ;
Gynomonœcism—ray and disc florets of Compositæ.

Irritability

354. Stigma. Using recently cut and carefully gathered blooms of *Mimulus luteus*, touch the inner surface of one of the stigmatic lobes with a slender pencil point or sharpened match end. Note the rapid closing of the two lobes and their recovery to the open position in about half an hour. Repeat the stimulation, using a point which has upon it some pollen from the stamens, preferably from another *Mimulus* flower. Note that in this case the lobes remain closed.

If *Martynia*, *Torenia* or *Mimulus cardinalis* (not *M. luteus*) is available, fix a pair of cover-slip forceps in a small vice and arrange a flower so that the tip of one stigmatic lobe is held by the forceps. When the fixed stigma is stimulated by a touch, transmission takes place, and the other lobe moves towards the fixed one.

No stimulation is produced in any of these cases by a touch on the *outer* surfaces of the stigmata.

355. Style. Touch the style of *Arctotis aspera*, while it still retains pollen amongst the outer hairs ; note that the resulting movement is always towards the side which is touched. If the conditions are warm and sunny the capitula should be vigorous ; then the style will regain its original irritability in a very short time, while remaining in the position assumed by the first movement ; less than half a minute may be sufficient. Styles which have been withdrawn into the corolla, after the first, or male stage, are not sensitive to touch when they again project with spreading style branches. For details of the successive stages, see Small in "New Phytologist," 14, p. 216, 1915.

Stamens. The following experiments should all be carried out on warm, sunny days when the flowers will be in good condition for reacting to the stimulus of touch.

356. Using flowers of *Berberis* or *Mahonia* which have been recently cut and carefully gathered, note that when stimulation is applied by touching, the movement of each stamen in a flower is independent of the other stamens, and is always towards the centre of the flower ; there is no

transmission of the stimulus if the touch is carefully confined to one stamen at a time. The sensitive region can be located by using a fine bristle or mounted needle. Gentle touches anywhere on the stamen, except on the inner (adaxial) surface of the filament, produce no movement. These stamens recover their original irritability and their original position in five or ten minutes; this should be compared with the rapid recovery of irritability without return to the original position shown by the style of *Arctotis*, which is sensitive on all sides of the cylinder and not physiologically bilateral like the stamen of *Berberis*.

357. Electrical Stimulation. Using the same material as for Ex. 356, apply the wire terminals from an active induction coil to a flower of *Berberis*, one wire touching the flower stalk and the other touching the firm central stigma; all the stamens are stimulated and move.

358. Anæsthetic Effects. Similar flowers of *Berberis* which have been given a small dose of chloroform vapour, e.g. placed under a bell jar with five drops of chloroform for each litre of air capacity for ten minutes, show no irritability; but recover the ability to move when touched after being removed from under the bell jar.

359. Compositæ. Provided that the conditions are favourable, i.e. warm and sunny, five types of movement of the anther tube in response to the touch stimulus may be found amongst the Compositæ; thus the pollen is presented by the piston action of the young style with:

A. Downward movement of the whole tube, all the filaments contracting equally; stimulus transmitted and response simultaneous; e.g. in *Achillea cristata*, *Anthemis canescens*, *Coreopsis tinctoria*, *Erigeron grandiflorus*, *E. speciosus*, *Helipterum roseum*, *Matricaria chamomilla*, *Tanacetum vulgare*, etc.

B. Lateral movement of the tube, indefinite in direction; stimulus transmitted and response irregular; e.g. in *Arnica alpina*, *Centaurea aspera*, *Carduus tenuiflorus*, *Cirsium kernerii*, etc.

C. Lateral movement of the tube towards the touch, by the contraction of one filament; stimulus not transmitted to other filaments; this is the common type; e.g. in *Achillea* spp., *Bellis perennis*, *Catananche* spp., *Centaurea* spp., *Chrysanthemum* spp., *Cirsium palustre*, *Carduus crispus*, *Heliopsis*, *Lactuca*, *Lapsana communis*, *Saussurea*, some species of *Senecio*, *Solidago*, *Sonchus*, *Taraxacum officinale*, etc.

The contraction of one filament and bending of the others is particularly easily seen in *Lapsana communis*.

D. Lateral movement of the tube, always towards the centre of the capitulum; stimulus transmitted and response only in

the posterior filament, *e.g.* in *Centaurea cyanus*, *C. axillaris*, *Aster lipskyi*.

E. As C, but explosive in rapidity and pollen ejected for some distance ; *e.g.* in *Gerbera* spp., *Perezia multiflora*.

For details and other examples, see "The Origin and Development of Compositæ." Small, 1919.

CHAPTER XXII

FERTILISATION AND EMBRYOLOGY

360. Germination of Pollen. (a) Note the characteristics of pollen grains from a variety of plants. Place some pollen grains from each kind of plant in a watch glass with about ten drops of 5 per cent. cane-sugar solution. Set this preparation aside, covered with a beaker or bell jar, in a warm place. Examine the contents of the watch glass after three hours, under the low power of the microscope. Note the particular kinds of pollen which germinate well under these conditions. Mount a few examples with a cover-slip and draw two or three with the pollen tube showing. See (b) and (c) below.

(b) A similar preparation may be made at the same time, using 10 per cent. cane-sugar solution. Other kinds of pollen grains may germinate in this case, while the previous kinds will probably be less successful than before ; the latter may not germinate at all (see (c) below).

(c) Set aside the preparations from (a) and (b) overnight. The sugar solution concentrates by evaporation and further kinds of pollen may germinate.

The concentration of cane sugar necessary for ready germination of pollen varies with the species. It can be determined by experimenting with different solutions, but the following notes of favourable concentrations may be useful : primrose, sunflower, snowdrop, *Allium ursinum* and lily 5 per cent. ; wallflower 6 per cent. ; narcissus 7 per cent. ; bean, pea and bluebell (*Scilla nutans*) 10 per cent. ; *Fritillaria imperialis*, *Cynanchum officinale*, *Echeveria* spp. and stonecrops 15 per cent. ; *Rhododendron*, *Azalea* and other Ericaceæ 10 per cent. sugar with 1 per cent. malic acid.

361. Chemotropism of Pollen Tubes. (a) Using an appropriate cane-sugar solution and pollen, as determined by the results of Ex. 360, place one drop of solution on a slide with a "well" or glass ring, add pollen-grains and a small piece of stigma from the same flower. Grease the

edge of the well or ring, and fit a cover-slip. Set aside this preparation for about three hours, and note the converging of the pollen tubes towards the stigma. Bluebell pollen, with 10 per cent. cane sugar, is usually successful. This is an example of **positive chemotropism**.

(b) **Negative chemotropism** in pollen tubes may be demonstrated by mounting pollen grains in a drop of appropriate sugar solution on a slide *without a well*, and adding a cover-slip. The tubes may grow towards the centre, away from the greater oxygen supply around the edge of the cover-slip. *Fritillaria* and *Cynanchum* pollens are suitable; other pollen tubes may or may not show this negative chemotropism.

362. Pollen Tubes In Situ. (a) Examine and make line diagrams of the prepared sections showing pollen tubes germinating on the stigma, e.g. of *Ænothera*, *Eschscholtzia*, etc.

(b) Using mature flowers in which the stigmata are not yet withered pollen tubes may be found *in situ*, usually around the conducting strands of the style, by means of suitable manipulation and staining. Slender styles are mounted in water on one slide and squashed with another slide placed above; thin longitudinal sections are taken of thicker styles. In order to obtain good material it is frequently necessary to dust the stigmata with pollen one or two days before the material is required for study. A suitable stain for fresh preparations is brom-cresol purple for lily sections, crocus squash preparations, etc.

363. Fertilisation. Examine and draw, under H.P., prepared sections showing double fertilisation in *Lilium*, *Senecio*, or other available material (cp. Fig. T 750).

Embryology

364. Dicotyledon. (a) Remove and mount in water or dilute potash a number of ovules from fresh immature fruits of *Capsella bursa-pastoris*. Add a cover-slip and, under a dissecting microscope or good pocket-lens, press upon the cover-slip over one ovule at a time in order to get carefully made squash preparations. Examine your preparation under L.P. for embryos which lie outside the burst ovules. Repeat the process until a suitable series of stages is obtained. Draw a representative series of embryos under H.P. (see Fig. T 753).

(b) Similar squash preparations may be made of immature ovules from preserved material of many other plants, provided that the material has been preserved in "Dutch pickle,"¹ and that it is warmed in 0.5 per cent. potash for a few minutes when required for use.

365. Monocotyledon. (a) Repeat the above manipulation, using ovules from fresh immature fruits of *Alisma plantago*. Draw a representative series of embryos under H.P. (see Fig. T 755).

(b) Other ovules of monocotyledons yield similar squash preparations with the preservation and manipulation given in Ex. 364 (b).

366. Examine and draw under H.P. the prepared slides of abnormal and other embryos, e.g. *Eranthis*, *Ranunculus ficaria*, *Loranthus*, *Nymphaea*, *Geranium*, *Crataegus*, *Lilium*, also various orchids and Leguminosæ.

367. Pinus. Cut longitudinal slices of several seeds of *Pinus pinea* (pine-nut). At the tip of the radicle the large irregularly coiled suspensor is frequently to be found, persistent even in the mature seed. Make a line drawing of the structure when a good example has been obtained.

368. Polyembryony. Remove the seed-coat from an orange seed and, using a dissecting microscope, dissect the contents carefully. One or two obviously dicotyledonous embryos will be found, and also a varying number, up to twelve or more, of semi-translucent embryos which may be only partially differentiated. Draw to the same scale all the embryos found, to show the irregularities in shape and the relative development in the series.

¹ Dutch pickle is a mixture of equal parts of water, glycerol and ethyl alcohol.

CHAPTER XXIII

THE FRUIT ¹

MANY fruits are characteristic of certain families, in the study of which these examples will be considered. Dried plants in fruit are, however, very useful for a general survey of the varieties of fruits and of their classification. The examples given below have been chosen so that material may be either readily obtained, readily dried and stored for repeated use, or readily purchased in the fresh condition at the greengrocer's or fruit shop.

369. Examine carefully and make large-scale drawings to show the characteristic external and internal features of the ripe condition of the following fruits ² :—

Dehiscent Fruits

Follicle—*Aconitum*, *Delphinium*, star anise (*Illicium*) ;

Legume—*Vicia*, *Pisum*, *Cytisus*, *Ulex* ;

Siliqua—*Cheiranthus* ;

Silicula—*Capsella*, *Lepidium*, *Lunaria* ;

Capsule—with dehiscence ;

 septicidal—*Hypericum*, *Scrophularia*, *Colchicum* ;

 loculicidal—*Viola*, *Iris* ;

 septifragal—*Digitalis*, *Datura* ;

 dentate—*Lychnis*, *Silene*, *Dianthus* ;

 porous—*Papaver* ;

 valvular—*Campanula*, *Antirrhinum* ;

 by lid, pyxidium—*Plantago*, *Anagallis*, also prepared specimens of *Hyoscyamus* and *Lecythis*.

¹ The elementary study of the contents of this chapter is mainly more suitable for school or nature-study work ; but it is included here in order to preserve the continuity of the exposition and to indicate what practical knowledge the university student should have, as a background for the theoretical consideration of the subject.

² At least one example of each group should be used.

Indehiscent Fruits

- Achene—buttercup ;
 Caryopsis—wheat, maize ;
 Cypsela—sunflower, scabious, valerian ;
 Samara—ash, elm ;
 Nut—acorn, hazel, sweet chestnut ;
 Nutlets—dock, buckwheat.

Schizocarpic Fruits

- Double samara—sycamore ;
 Lomentum—pea nut, cassia pod, senna pod ;
 Cremocarp—*Myrrhis*, *Heracleum*, hemlock, aniseed, caraway,
 cummin, coriander, fennel ;
 Carcerulus—deadnettle, comfrey, mallow ;
 Regma—cranesbill, spurge, *Ricinus*.

Succulent Fruits

- Berry—grape, red and black currant, *Solanum dulcamara*,
S. nigrum, *Atropa Belladonna*, orange, tomato ;
 Pome—apple ;
 Receptacular—strawberry, rose-hip ;
 Drupe—cherry, elderberry, pickled walnut and olive ; also—
 with fibrous sarcocarp—sweet almond, coconut ;
 Drupel—blackberry, raspberry ;
 With pyrenæ—holly, ivy.

Pseudocarps

Mulberry, pineapple, fig.

Many of the above fruits are figured in the Textbook, and further details of nuts and other fruits may be found in “Pocket-lens Plant Lore:”

CHAPTER XXIV

SEED DISPERSAL ¹

370. REMOVE all the one-seeded fruits (cypselæ) from a single capitulum of *Calendula officinalis* or *Dimorphotheca* spp.; classify the fruits according to their degree of suitability for dispersal away from the parent plant. Draw the extreme types and at least one intermediate type.

371. Examine the mature structure of the fruits or seeds as given below; sketch all examples supplied and label your drawings with the name of the plant, the name of the part or parts dispersed naturally and the morphological nature of the dispersing mechanism.

Wind Dispersal

Light seeds—*Pyrola*;

Censer—campion, poppy;

Winged seeds—pine and prepared specimens of wooden pear (*Xylomelum*);

Winged fruits, with extension of—

Pericarp—ash, sycamore, birch;

Perianth—dock, sea pink, sea lavender;

Calyx—sage;

Bracts—hornbeam, hop;

Balloon—*Physalis*, *Orobus*;

Rolling—prepared specimen of *Anastatica*;

Parachute mechanisms—

Hairy seeds—willow, willow herb, *Strophanthus*;

Hairy styles—clematis, alpine anemone;

Pappus—dandelion, groundsel.

372. Experimental Wind Dispersal. The experimental demonstration of the relative efficiency of the various structural

¹ The study of the more elementary structural aspects of seed dispersal is more suitable for school or nature-study work; but some notes are included here in order to preserve the continuity of the exposition and to indicate what practical knowledge the university student should have, as a background for the theoretical and experimental consideration of the subject.

arrangements which *appear* to be suited for wind dispersal of seeds may be carried out with the following apparatus :

The Wind Channel. This may be merely a wide glass tube, or a more efficient wind channel may be made of three 6-foot lengths of $\frac{1}{2}$ -inch timber arranged with a glass front to produce a square tube 6 feet long and 6 inches wide. In the latter case the inside angles of the tube should be rounded off by suitable wooden slips (see Fig. 26A); the wooden parts of the interior should be painted white.

The Fan. An electric fan, suitably guarded, may be used as the source of wind; the strength of the air movement being controlled by varying the speed of the fan and also by changing the distance between the fan and the entrance to the tube (see Fig. 26, B).

The Anemometer. A 3-inch two-dial Biram Anemometer has

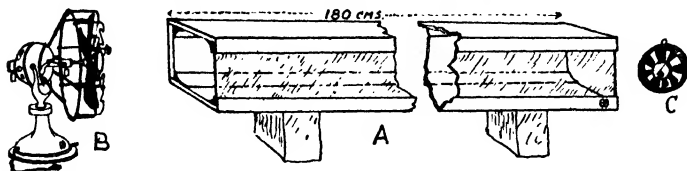


FIG. 26.—Wind Dispersal Apparatus. A, wind channel; B, electric fan; C, Biram Anemometer.

been found convenient in practice. It can be placed well into the larger wind channel to obtain the reading of wind velocity.

Using the above apparatus determine the *minimum* velocity of wind necessary to blow at least one of each of the larger types of seeds or fruits completely through the wind channel. Each specimen should be held in a pair of tweezers or on a slide at least 2 inches inside the entrance to the wind channel, and liberated carefully. The anemometer should be used after the critical velocity has been reached; intermediate values have an interest only in more advanced work. Tabulate your results to show the relative efficiency of the various structural modifications in the examples which you study.

373. (a) Determine the rate of fall in quiet air of a few of the seeds and fruits used for Ex. 372, using, if possible, *the same specimens* of each as are used in Ex. 372. This may be done by timing, with a stop watch, the fall of a single seed or fruit through a distance of about 15–20 feet. Shorter distances increase the errors of timing and longer distances make it difficult to see the specimen at the beginning of its fall. The raising and dropping present some difficulties in practice, but if a quiet room such as an

unoccupied lecture theatre or laboratory be available with a horizontal beam or pipe at the required height, the simple apparatus shown in Fig. 27 is quite effective. The tilting tray T has a perfectly clean microscope slide stuck to the top of the metal so that one end of the slide projects a few millimetres beyond the metal. The tray is

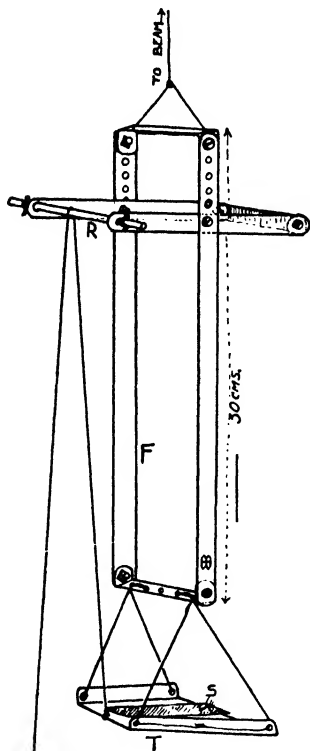


FIG. 27.—Dropping Apparatus (for explanation see text).

beyond the metal. The tray is slung by thin cord from its corners to the base of the rectangular frame F above. The rectangle carries two cross-beams, connected at one pair of ends by a flat bar and at the other end by a cylindrical rod R. A thread, attached to one end of the tray, carried over the rod and down to the floor, enables the operator to tilt the tray. A few "Meccano" parts, thin cord and thread are all that is required for the making of this apparatus. A piece of cord, about 40 feet long, is attached by one end to the top of the rectangular frame, the rest is coiled and thrown over an overhead beam. The tray can now be steadied, a seed or fruit, S, laid near the outer end of the glass slip and the whole pulled up as far as is convenient. With the stop watch in the hand, the thread can be pulled and the tray tilted, while the beginning of the fall is watched from below. A sheet of *white* paper below, on the floor, facilitates the observation of the completion of the fall; and the seed should fall within the square metre directly below the apparatus if the air is quiet. Consecutive observations on the same fruit have given times varying only by a fifth of a second with only 10 feet of a fall, in the

case of pappose parachute fruits. The actual time for a 20-foot fall varies from one to twenty seconds with different kinds of wind-dispersed seeds.

(b) From the above results calculate the minimum velocity of wind for dispersal, using the formula $w = \sqrt{2 F^2} + \sqrt{0.25 F^2}$.

Compare the values for w obtained in this way with the

experimental values as determined in Ex. 372 (see the Textbook, pp. 335-336).

374. (a) Study under a good pocket-lens the movement of the pappus hairs in dandelion, groundsel, coltsfoot and *Centaurea* when the relative humidity of the atmosphere is changed. A bell jar with an open dish of water may be used to get a damp atmosphere, and drying near a stove or over a small flame to get the opening movements which occur under drying conditions.

(b) The relative humidity may be determined with reasonable accuracy by the wet-and-dry-bulb thermometric method, or the readings of the Baro-Thermo-Hygrograph may be taken as more convenient, if the instrument is placed under the bell jar for damp conditions and in a warm dry glasshouse for dry conditions. Determine the critical relative humidity for the opening or closing of (i.) the pappus of a papose fruit ; (ii.) the teeth of a dentate capsule, e.g. *Stellaria media* or similar thin-walled fruit, and (iii.) a valvular capsule, e.g. snapdragon.

Water Dispersal

375. Set aside, in corked specimen tubes half-filled with water, ten seeds each of onion and yellow flag or ten fruits each of *Anthriscus sylvestris* and *Cenanthe crocata*. Observe daily and note each day the numbers of each kind of seed or fruit which have sunk below the surface of the water. When you have a distinctive record leave the seeds as they are for one more week and then test their germination capacity, as in Ex. 22.

Other pairs of land plants and water-side or water plants may be treated in the same way. Any convenient material should be used.

376. Examine and make a line drawing of a natural coconut cut in half longitudinally (Fig. T 838). Note the prepared specimens of *Entada scandens*, *Cesalpinia bonducella*, *Raphia*, etc.

Animal Dispersal

377. (a) Examine the fruit of the mistletoe ; mount a little of the fresh viscin and observe its structure under the microscope. Cut the seed in half longitudinally ; note and draw the dark green embryo.

(b) Examine and draw, under a good pocket-lens or the low power of the microscope, the hooks on some of the following :

Geum, *Medicago*, *Daucus*, *Galium aparine*, *Acaena*,
Harpagophytum, *Bidens*, *Agrimonia*, *Arctium*,
Xanthium.

Annotate your drawings to indicate the morphological part which bears the spines, e.g. style, pericarp, pappus setæ, calyx, involucre, etc.

Projection Mechanisms

378. Note and draw the fresh or prepared specimens of:—
Dorstenia (turgid); *Ulex*, *Lupinus* (torsion); *Geranium*
or *Erodium* (sling); *Viola* and *Illicium* (valvular
compression).

Resting Periods

379. (a) Revise the experiments done with willow and coltsfoot, as examples of seeds having a negligible resting period combined with rapid loss of viability (see Ex. 210).

(b) Collect sixty fresh seeds of *Limnanthes douglasii*. Test the germination capacity at once, using twenty seeds (see Ex. 22). Keep the others dry; test their germination capacity after one month and after six months or a year, using twenty seeds for each test.

(c) The natural length of the resting period varies and in many cases there is a minimum period during which the seeds do not germinate under the usual conditions. Collect from the plants at the appropriate season mature seeds, and test the germination capacity as soon as possible. Test the seeds which do not germinate readily in the "new" condition after :

- (i.) soaking overnight in N/100 sulphuric acid; or, better, 5 per cent. solutions of citric, tartaric or malic acid;
- (ii.) exposure to chloroform vapour for not more than four hours;
- (iii.) scratching the seed-coats with a strong sharp point;
- (iv.) heating for three hours at 35°–40° C.

Suitable sources of new seed are for (i.) cereals, flax, lentils, broad bean, apple, hemp; (ii.) *Phaseolus vulgaris*, white lupin, rape, apple; (iii.) wild vetches, clovers, gorse, broom; (iv.) wheat, rye.¹

¹ Oats require a low temperature (about 5°–10° C.) when fresh.

CHAPTER XXV

PERMEABILITY AND ASSOCIATED PHENOMENA

Emulsions

380. (a) Examine a drop of milk under the microscope, L.P. and H.P.

(b) Mix one drop of milk and one drop of Sudan III. ; examine under H.P., and note the oily internal phase.

General Rules for Emulsion Phases

I. The medium which wets an insoluble emulsifying agent or dissolves a soluble emulsifying agent becomes the external, continuous or dispersion phase, with the other medium as the internal or dispersed phase.

II. Emulsions can be largely diluted only with the medium which occurs as the continuous phase.

381. (a) Place 2 c.c. linseed oil or olive oil coloured *strongly* with Sudan III. in each of two test tubes ; add 2 c.c. of 1 per cent. solution of sodium hydroxide to one and 2 c.c. of lime water to the other ; shake both tubes vigorously.

Examine the resulting emulsions under L.P. without a cover-slip, and note the oily internal phase when the mono-valent alkali is used, forming a soap soluble in water, but not in oil ; also the oily external phase when the divalent alkaline calcium is used, forming a soap soluble in the oil, but not in water. The first is an oil-in-water emulsion and the second is a water-in-oil emulsion.

(b) Take about 1.5 c.c. of each emulsion in separate test tubes, and note the behaviour of each when diluted gradually, shaking after each dilution, (i.) with water and (ii.) with oil.

(c) Using xylol and water in equal parts with lampblack and with barium sulphate respectively, test rule I. above for insoluble emulsifying agents " wet " by xylol or by water.

Note. These exercises give useful ideas on a large scale of possible minute structures for cytoplasm.

382. Suspensions. Prepare a suspension of resin by

rubbing a piece of stick gamboge in an evaporating basin with 1 or 2 c.c. water, adding 10 c.c. water and decanting the yellow liquid.

(a) **Brownian Movement.** Mount a drop of this suspension with a cover-slip and examine carefully under H.P. Note the dancing movements of the smaller particles of about 1 micron diameter, and the slower movement of the large particles, up to 5 microns diameter.

(b) **Cataphoresis.** Mount another drop of the suspension under a cover-slip on a slide fitted with electrodes and attached to a dry battery. Observe movement of the charged particles in one direction. Which electrode lies in this direction and what is the sign of the charge on the particles?

(c) Advanced students should use the method in (b) with protein suspensions, e.g. casein, in acidic and alkaline media in order to understand "isoelectric points of amphoteric substances."

Colloidal Solutions

383. Prepare a colloidal solution of ferric hydroxide by one of the following methods :

(a) To 10 c.c. 30 per cent. solution of ferric chloride add 0.880 solution of ammonium hydroxide, drop by drop, stirring, until an abundant precipitate is formed; add dilute hydrochloric acid carefully, drop by drop, stirring until just sufficient has been added to dissolve the precipitate. Transfer this liquid to a small dialyser suspended with its membrane 2 cm. below the surface of about 500 c.c. water. Change the water *below* the dialyser until it gives no colour with potassium ferricyanide. Leave for two days with at least two changes of water.¹ The red liquid (*dialysate*) retained in the dialyser is a liquid colloidal solution (*sol*) with many *emulsoid* properties. *Suspensoids* are distinguished from emulsoids in that the disperse phase is usually solid, the concentration and viscosity low; and precipitation is obtained with relatively small quantities of electrolytes or oppositely charged sols. Ferric hydroxide sol has a solid disperse phase and is precipitated by relatively small quantities of electrolytes, but in other respects it is emulsoid; on standing, the viscosity may rise so high that a semi-solid or solid colloidal solution (*gel*) may be obtained. The ferric hydroxide sol has a positive charge and protects suspensoids from precipitation, e.g. it may give no actual precipitate on addition of one drop of 1 per

¹ If the precipitation and solution are successful the water outside will contain only ammonium chloride with traces of iron.

cent. sodium chloride to 10 c.c. sol, followed by silver nitrate solution.

(b) To 250 c.c. rapidly boiling water add 3 c.c. of 10 per cent. ferric chloride, gradually with continuous stirring. The resulting clear reddish-brown solution has colloidal properties similar to those of the dialysate above.

384. Examine the sol obtained in Ex. 383, thus :

(a) To separate 1 c.c. portions add—

(i.) One cubic centimetre 5 per cent. solution of sodium chloride ; note resulting precipitation.

(ii.) One cubic centimetre 1 per cent. solution of sodium chloride, shake well ; note absence of precipitation.

(b) Set aside the remainder and look for increasing viscosity at weekly intervals.

Other colloidal solutions include methylene blue, Bismarek brown, albumin and agar-agar with a positive charge, also silver, gold, starch, resin, tannin and lecithin with a negative charge.

Adsorption

Emulsification, as in Ex. 381, involves the adsorption of the emulsifying agent by the disperse phase in which it is insoluble. This adsorption may show polarity, as in liniment of turpentine where the arrangement is said to be as follows :

	hydrophobous	hydrophilous	
turpentine globule :	$\text{CH}_3\text{---R}$	---COOK	water.
	soap film		

Other examples of adsorption may be studied in the following exercises :

385. To 10 c.c. of water in a test-tube add one drop of 1 per cent. solution of methylene blue ; shake and note the tint ; keep 5 c.c. for comparison and to the rest add about 1 mg. of saponin, or a few fragments of soap-bark, and shake vigorously. Note the paler tint of the liquid beneath the blue foam. Decant the liquid, and add a few drops of alcohol to the foam ; note the deeper blue of the now more concentrated solution of methylene blue. This is an example of mechanical adsorption by the film surfaces of a foam and applies equally with acidic or basic water-soluble dyes.

386. Upon a filter paper, held horizontally in the air,

drop separate drops of aqueous solutions of methylene blue or basic fuchsin and of erythrosin, indigo-carmin or acid fuchsin. Note that the first (basic) dyes are adsorbed by the negatively charged paper giving a deep stain in the centre, surrounded by a watery unstained zone; while the second (acidic) dyes are not adsorbed to the same extent and give a stain which is more uniformly distributed over the area wetted by the water. The coloured basic ions of methylene blue are adsorbed strongly by reason of the different electric charges of these and the paper. The coloured acidic dyes are adsorbed mechanically to a certain extent, but are repelled towards the water by reason of the similar sign of the charges of the coloured acidic ions and the paper; the watery zone is therefore narrower.

387. (a) Since filter paper behaves as if it is acidic, the degree of "active acidity" may be determined by using the drop method, as given above, with a series of indicator dyes at or about their various neutral points. Using carefully balanced solutions,¹ drop on to a horizontally held filter paper single separate drops of at least two from each of the following groups of aqueous solutions of indicators: (1) neutral red, phenol red, brom-thymol blue, brom-cresol purple, diethyl red; (2) methyl orange, methyl red, benzene-azo-*a*-naphthylamine, brom-cresol green, brom-phenol blue. From your observations deduce the degree of base avidity of the acidic filter paper (see Ex. 402).

(b) If the whole series of indicators can be used very interesting transitions will be found. Brom-cresol purple is anomalous in its behaviour; but with the others "acid" colours are given by NR, PR, BTB and DER, "alkaline" colours by MO, MR, BAN, BCG and BPB; while adsorption, as measured by the relative width of the watery unstained zone, decreases gradually from the DER point to a minimum with BCG, or to zero with BPB which shows sharp coloured edges. BCP behaves like BPB. There is thus an intermediate region with adsorption, but alkaline colours for MR, BAN and to a very small extent with BCG. This intermediate region extends from *pH* 5.6 to about *pH* 4.4, a comparatively narrow range.

388. Reversal of Charge. (a) Repeat Ex. 386, after

¹ These may be obtained by adding centinormal solution of hydrochloric acid or sodium hydroxide to 10 c.c. of the indicator solution until the colour is intermediate between the acid colour and the alkaline colour.

wetting the filter paper, (i.) with 5 per cent. solution of sodium chloride, (ii.) with centinormal hydrochloric acid.

(b) Repeat Ex. 387 with fragments of gelatin in 5 c.c. quantities of distilled water. Determine the "base avidity" of gelatin.

(c) Prepare six tubes, two with 10 c.c. each of centinormal hydrochloric acid, two with 10 c.c. 5 per cent. sodium chloride; and two with 10 c.c. tap water. Drop a few fragments of gelatin into each tube, and add a few drops of 1 per cent. aqueous methylene blue to one set of three tubes, and a few drops of 1 per cent. aqueous erythrosin to the other set of three. Record the degree of staining or non-staining effects, after washing the gelatin fragments with three changes of tap water.

From Exs. 387 and 388 (b) it will be seen that both filter papers and gelatin in contact with water behave as if they were weak acids. From Ex. 388 (a) and (c) it will be seen that the surface charge and staining properties of these substances are reversed in contact with neutral salts or dilute mineral acids.

389. Write a series of brief notes on the staining properties of cell-walls and cell-contents as observed in your other studies, connecting the observed staining reactions with the basic and acidic dyes used, and giving some account of the differentiation of tissues and cell-contents in relation to their adsorption of these dyes.

Permeability

390. Barger's Method for Osmotic Pressure. Suitable material for these studies may be found in sunflower hypocotyl stripped of the epidermis, also rhubarb petiole, onion bulb scales, young bean stems, etc., with the outer layers removed.

A stock solution of anhydrous calcium chloride is prepared by dissolving 48.19 grammes of that salt in distilled water to make 1,000 c.c. of solution. This is isotonic with 1.0 molar sucrose solution taking sucrose I. C. 1.88 and calcium chloride I. C. 4.33. By appropriate dilutions a series isotonic with 0.10, 0.20 and 0.30 molar sucrose is prepared. Three clean¹ capillary tubes of about 1 mm. bore and of even width throughout are selected. The plant material is frozen on an ice-salt mixture; de-frozen; and the juice is then pressed out. By appropriate manipulation, successive alternate small drops of calcium chloride solution and plant juice with 2 or 3 mm. of air between the ends of the drops are arranged in the capillary tubes. A different concentration of calcium chloride is used for each tube. There should be at

¹ Dirty capillary tubes are useless for this purpose.

least three drops of plant juice in each tube. The tubes are then sealed in a small flame and attached by means of Canada balsam or soft paraffin to a microscope slide. The lengths of each drop of plant juice and of each drop, except the end drops, of calcium chloride are then measured with an eyepiece micrometer. The slide is set aside in a cool place, preferably in a Petri capsule, for twenty-four hours; after which the drops are again measured. In the lower concentrations the plant juice drops may be larger than initially and the salt solution drops smaller; while in the higher concentrations these changes may be reversed. The intermediate condition of no change in drop lengths may coincide with any of the concentrations, but if it lies between two of the series a half-way approximation may be sufficiently accurate. The student is advised to prepare a series of six concentrations in the critical range, e.g. 0.20, 0.22, 0.24, 0.26, 0.28, 0.30, for an intermediate showing increase in the sap drops with 0.20 and decrease with 0.30; and to repeat the preparation of tubes and measurement of the drops. This will give an accuracy which is suitable for most practical purposes. Using the table on p. 63, express the critical concentration in atmospheres of pressure.

By this method the *physical* or *theoretical osmotic pressure* of a plant juice can be determined.

There are other methods, such as depression of the freezing point determined for small quantities in a micro-Beckman apparatus, also various electrical methods, but these are only suitable for more advanced work.

391. Effective Osmotic Pressure. Using the same source of plant material as for Ex. 390, determine the *effective osmotic pressure* of the cell sap by the microscopic plasmolytic method, using cane sugar solutions as in Ex. 133. Since sucrose penetrates very slowly, if at all, any difference between the *physical osmotic pressure* as determined in Ex. 390 and the *effective osmotic pressure*, as determined above, may be attributed to exosmosis of the plant sap constituents. Equilibrium may not be established until after a long period of immersion, but an approximate standard of reference is obtained by using the twenty- or thirty-minute interval and the 50 per cent. of plasmolysed cells, in the determination of the critical concentration of sugar for plasmolysis. Using the table on p. 63, express the critical concentration in atmospheres of pressure.

392. Suction Pressure. Using the same source of plant material as in Ex. 390–391, determine the suction pressure of the tissue being studied, applying the Molz method as in Ex. 135. The cutting of slices only 1 mm. long may be found difficult, but the process is relatively easy, if a slice of the tissue be cut about $1\frac{1}{2}$ mm. *thick*; this slice is then cut in thinner sections in the vertical direction *AB*, and these in their turn are cut into pieces *xy*., about $1\frac{1}{2}$ mm. long, with a thickness equal to that of the set of

thinner sections and any convenient width (see Fig. 28). Using the table on p. 63, express the critical concentration in atmospheres of pressure.

393. In the three preceding experiments determinations are made of—

- (i.) the physical osmotic pressure— P^b ;
- (ii.) the effective osmotic pressure— P^c ;
- (iii.) the suction pressure— S .

(a) Since $S = P^c - W$ (the wall pressure, see p. 62), and

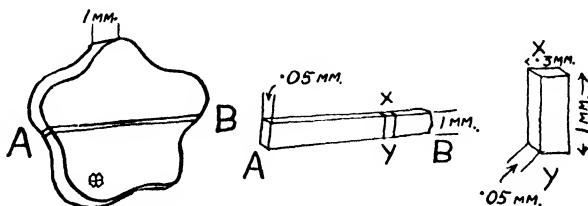


FIG. 28.—The large slice is about $1\frac{1}{2}$ mm. thick ; from this is cut a thin section AB about .05 mm. thick ; and from this section parts such as xy about 0.3 mm. wide are cut, (see text).

both S and P^c have been measured in the same uniform material we can calculate W , the wall pressure or turgor pressure.

(b) Since any excess of P^b over P^c can be attributed mainly, if not entirely, to the exosmosis of sap, we may take P^m as the pressure lost on account of the permeability of the protoplast to the cell sap constituents.¹ Then $P^m = P^b - P^c$ and the formula for permeability coefficients $\mu = 1 - \frac{K \text{ obs.}}{K \text{ theoretical}}$ (Textbook,

p. 348) can be applied as $\mu = 1 - \frac{P^c}{P^b}$ (under the specified conditions).

(c) Provided that the changed conditions do not effect the cell wall directly, we can therefore subject the tissue studied to various experimental treatments such as immersion in weak acids or weak alkalies or solutions of neutral salts (see Ex. 386-388),

¹ Stiles (*loc. cit.*, p. 172) criticises the application of Tröndle's permeability coefficient formula to external solutes, but Lepeschkin found some correspondence between his values and those determined by the permeability "factor" or coefficient method. In any case the same criticisms do not apply to measurements of the permeability of the protoplast to internal solutes with a large quantity of external water.

or to light and darkness, or to varying temperatures, etc., and redetermine the data for P^B , P^e and S . The wall pressure W changes with P^e and the values in the equation $S = P^e - W$ give a check upon any change in the cell wall; while the values in the equation $P^{in} = P^B - P^e$ enable us to detect variation in the permeability coefficient of the protoplasts of particular tissues, such as epidermis, cortex, etc.

Example. If, under normal conditions, we have $S = 3.4$ atm., $P^B = 8.2$, $P^e = 6.5$, then $W = 3.1$ and $\mu = 0.21$. While, under experimental conditions, we may have $S = 2.6$ atm., $P^B = 7.9$, $P^e = 5.2$; then $W = 2.6$ and $\mu = 0.34$. It will be noted that here μ is increased by 62 per cent., while P^e is reduced only by 20 per cent. and W by 16 per cent.

394. Negative Osmosis. Set up the thistle-funnel apparatus as in Ex. 122, using capillary or other narrow-bore tubing with a short-stemmed thistle funnel and a pig's bladder membrane. Fill the thistle funnel with an aqueous $M/2$ solution of citric (or tartaric) acid; attach the narrow-bore tubing and place the membrane below distilled water. Note the movements of the column of acid solution (a) during the first four hours and (b) the following day.

395. Fill two U-tubes half-way up each arm with 3 per cent. gelatin, and allow these to set. (a) Take one U-tube and add solutions of Congo red to one arm and eosin solution to the other. Set aside for twenty-four hours and note during the following week the slow diffusion of the large congo red molecules as compared with the more rapid diffusion of the smaller eosin molecules. (b) Take the other tube and add 1 per cent. solution of potassium ferrocyanide to one arm and 1 per cent. copper sulphate to the other. Set aside for twenty-four hours or longer and, from the position of the brown membrane of copper ferrocyanide, compare the rates of diffusion of the copper and ferrocyanide ions.

HYDROGEN-ION CONCENTRATION

N.B. Water throughout these experiments should be *distilled* water. All apparatus and glass must be clean.

396. Take two test-tubes and, by means of a narrow strip of paper gummed along the length of each, graduate the tubes at 1 c.c. and at 10 c.c.

(a) **Strong Acid.** Take 1 c.c. $N/1$ hydrochloric acid (*ca.pHo*); add five drops of brom-cresol green and note the colour; dilute to 10 c.c. with distilled water and note the colour of the $N/10$ acid; take 1 c.c. of the $N/10$ dilution,

add five drops of brom-cresol green and 9 c.c. water ; note the colour of the N/100 acid ; repeat this 1 in 10 dilution, adding five drops of indicator to the 1 c.c. quantities at each stage for N/1000, N/10,000, N/100,000 and N/1,000,000 concentrations. Reserve the 9 c.c. remainders in separate tubes ; compare the colours and tints throughout the diluted series, which approximates to hydrogen-ion concentrations corresponding to pH 1, 2, 3, 4, 5 and 6 respectively. Hydrochloric acid may be taken as completely dissociated and a strong acid.

(b) **Weak Acid.** Take 1 c.c. N/1 acetic acid ; add five drops of brom-cresol green and note the colour ; dilute to 10 c.c. with water and proceed as above to N/1,000,000. Compare the colours and tints obtained one with another ; then compare this series with the one for strong acid, particularly as regards the normality of the concentrations of the two acids which give the same or similar tints with the brom-cresol green. Acetic acid dissociates only to the extent of 0.42 per cent. and is therefore $\frac{98}{0.42}$ or 233 times weaker than aqueous hydrochloric acid of corresponding normality ; but the dissociation is higher at great dilutions.

(c) The above comparisons should be carried out with the same acids and brom-phenol blue in order to see more clearly the differences in the higher concentrations.¹ Other aqueous solutions of acids such as malic, citric and oxalic acids can be compared in the same way.

397. Buffers. Since normal biological acidities lie within the range pH 3 to pH 8, important biological buffer systems are those which are most effective in that range. These include mainly weak acids mixed with their salts, *e.g.* in plants there are phosphate, malate, citrate, oxalate and bicarbonate-carbonic acid systems. The salt systems of polyvalent acids may be said to control or buffer the change in hydrogen-ion concentration, when acid or alkali is added, by transferring ions from the less acid to the more acid salt or *vice versa*, thus the system $KH_2PO_4 - K_2HPO_4$ possesses the power of absorbing added H ions by increasing the proportion of the first salt in relation to the second, and thus preventing a change in the concentration of *free* hydrogen ions. The salt-acid systems of monovalent acids, such as acetic acid,

¹ Increased dissociation in the higher dilutions may prevent the demonstration of these phenomena with methyl red and indicators with higher change points.

are less easy to understand, but illustrate the fundamental principle better. All acids dissociate in such a way that--

$$\frac{[\text{H}^+] \times [\text{Ac}^-]}{[\text{HAc}]} = K_a \therefore \frac{1}{[\text{H}^+]} = \frac{[\text{Ac}^-]}{K_a[\text{HAc}]}$$

Since K_a is a constant (the dissociation constant) the concentration of H^+ varies inversely as $\frac{[\text{Ac}^-]}{[\text{HAc}]}$. The alkali salts of weak acids dissociate much more strongly than do the acids themselves ; therefore if a salt NaAc be added to an aqueous solution of the acid HAc , the concentration $[\text{HAc}]$ may remain the same, but the concentration $[\text{Ac}^-]$ is increased, thereby increasing the value of $\frac{[\text{Ac}^-]}{[\text{HAc}]}$ and of $\frac{1}{[\text{H}^+]}$, which means a decrease in the value of $[\text{H}^+]$, the concentration of hydrogen ions ; the Na^+ ions replacing the H^+ ions which become undissociated.

(a) Add to 10 c.c. of N/100 acetic acid, strongly coloured red with methyl red, about 1 gramme of pure (neutral) sodium acetate. Observe the alteration in colour indicating a considerable decrease in the hydrogen-ion concentration or increase in $p\text{H}$.

(b) Repeat using N/10 acetic acid strongly coloured yellow with brom-cresol green. Note that the change point of this indicator is lower and therefore N/10 acetic acid gives an alteration of colour with BCG, but not with methyl red, on the addition of the neutral salt.

(c) Prepare two very clean test-tubes ; half fill each tube with freshly distilled water ; add to each three drops of phenol red or brom-cresol purple. Add to one tube one drop of N/100 hydrochloric acid, and to the other tube one drop of N/100 sodium hydroxide. Compare with (d) below.

(d) Add to 5 c.c. of one-tenth molar K_2HPO_4 , coloured with five drops of phenol red, sufficient 0.1 M KH_2PO_4 to give a neutral tint, about $p\text{H}$ 7.0 or neutrality. Dilute the mixture with water to fill the tube. Transfer half to another clean tube, and add acid and alkali as above. There is no change of colour ; no large alteration in $p\text{H}$. Keep adding acid and alkali respectively to the two tubes, drop by drop, until the same colour change as in (c) is obtained. Compare the resistance to change of $p\text{H}$ in the phosphate buffer solution with the absence of any such phenomenon where distilled water alone is used.

N.B. Tap water is more or less buffered, and the above comparison cannot be made satisfactorily unless the water is recently distilled.

Anthocyan Indicators

398. (a) Immerse two sets of coloured petals or complete corollas in (i.) N/10 acetic acid, (ii.) ammonia (1 in 10 of 0.880 solution). Note and record the colour changes. Suitable materials can be obtained from most bluish Boraginaceae, such as comfrey, alkanet, lungwort, forget-me-not, etc., also *Scabiosa*, *Geranium*, *Pelargonium*, *Viola*, *Primula sinensis*, *Hyacinthus* (red or blue), *Scilla*; rose petals and cornflower corollas,¹ red beetroot, red radish, red cabbage, bilberry fruits, green and brown broad bean seeds, white and yellow maize fruits may also be used. Natural anthocyan indicators are common, and give rise to many colour changes as flowers mature, as in the Boraginaceae.

(b) Prepare a series of buffer solutions of pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, either by using the Universal Buffer Mixture (B.D.H.), or by Sørensen's citrate mixture (see Clark's "Determination of Hydrogen-ions"). Immerse petals or corollas from two or three of the above plants in each buffer solution and determine approximately the natural hydrogen-ion concentration of the cell sap. Closer approximations can, of course, be obtained by using smaller steps in the buffer series.

Natural and Other pH Data

399. Cut a healthy potato tuber in half with a clean, sharp knife. Place a few drops of carefully balanced diethyl red on one cut surface and a few drops of a similar solution of brom-cresol purple on the other. Note the colours.

(a) Draw a sharp razor edge gently across each surface. Note that the track of the cut shows yellow with BCP, and may not be visible with the DER.

(b) Then draw the back edge of a knife blade forcibly across each surface. A wider yellow track shows with the BCP, and a bright red scar with the DER.

The deep cut in (b) gives rise to the abundant carbon dioxide of injury and considerable acidity, while the gentle cut in (a) merely opens one layer of cells and allows the sap to escape.

400. (a) Extend Ex. 30 (b), using a young sprouting potato bud and as many indicators as available, e.g. BTB, BCP, DER, MR, BCG and BPB. Determine the range within which lies the natural hydrogen-ion concentration of the cell sap in the various tissues of the young stem. Observe the tissues under

¹ See Buxton and Darbishire, *Journal of Genetics*, 25, 71, 1929.

L.P. and note the changes in colour with BCP and DER with and without a cover-slip.¹

(b) Apply this Range Indicator Method to transverse sections of sunflower hypocotyl and older stem, and such other herbaceous material as may be convenient.² Examine the tissues under L.P. without a cover-slip; see Appendix II. for change points.

401. (a) Immerse a sliver, with stomata and guard cells, from a leaf in 0.04 per cent. aqueous diethyl red; set aside for two hours in darkness. Expose to bright sunlight and note the disappearance of the red colour from the guard cells due to a reduction of the carbon-dioxide concentration by photosynthesis. Note the variations in dull light with and without a cover-slip over the sliver on a slide, compare with Ex. 400 (a). Suitable material may be obtained from either side of a leaf of carnation, daffodil, bluebell or leek or the under side of a leaf of *Aucuba*, *Convallaria*, *Lilium* or *Tradescantia*.

(b) Using a potted plant of *Limnanthes douglasii* which has been kept in the dark for twenty-four hours before this experiment is begun, infiltrate a leaf segment with aqueous brom-cresol purple, by centrifuging the leaflet and solution together. Observe the colours quickly under L.P., and expose the leaflets in a little water to strong sunshine. Note the appearance of a bluish colour, indicating decrease of carbon dioxide and of intercellular acidity, with increase in photosynthesis.

402A. Base Avidity. Since the lignified or suberised walls of plant cells are stained by the acid colours of some indicators and not by others, while the substances of these walls are not ionised like water-soluble substances, the student should appreciate the meaning of the term "base avidity." Fatty acids combine with alkalies and thus show the presence of ionisable hydrogen and other ions. The same may occur with lignin and suberin. Compare Ex. 387 above.

(a) Shake up with aqueous methyl red or diethyl red, and other indicators if convenient, 1 c.c. quantities of liquid or melted, fatty acids such as myristic, palmitic, stearic, oleic and tiglic acids. Note the colours given by the fatty acid.

(b) Proceed as above after adding alcohol to the indicators used. The acid colours when present show throughout the liquid and are not, therefore, merely adsorbed as in Ex. 387.

¹ These are due to carbon dioxide accumulating under the cover-slip and escaping in the other case.

² The degree of differentiation which may be expected in most common materials as well as many other kinds of pH data, will be found in "H.i.c. of Plant Cells and Tissues" (see below).

Advanced students will find many instructive experiments on surface tension and colloids in relation to hydrogen-ion concentration in "An Elementary Course in General Physiology," by G. W. Scarth and F. E. Lloyd, (Chapman and Hall.) Other useful books, in addition to those mentioned in the Textbook, are those by Haas and Hill, Hatschek, Bayliss ; also "Hydrogen-ion Concentration in Plant Cells and Tissues." (Small, Borntraeger.)

CHAPTER XXVI

PLANT MOVEMENTS

INTRACELLULAR MOVEMENTS

Protoplasmic Streaming

402B. Tradescantia. When the material is available, observe the streaming of the colourless granular cytoplasm in various directions through the coloured sap in the staminal hairs from fresh flowers of *Tradescantia*.

403. Elodea. Mount a single leaf of *Elodea* in water, so that the under side is turned upwards, add a cover-slip; examine the cells under L.P. for intracellular movement. This may be found near the midrib when the leaf is otherwise quiescent. Under H.P. fix your attention on one particular chloroplast and determine the speed of rotation.

Movement may be initiated in cold weather (a) by roughly pulling a leaf off, (b) by gently warming the mounted leaf over a small flame, (c) by leaving the whole leafy stem overnight in a beaker of water together with two pennies or other copper coins.

404. Determine the effects of various agents upon the speed of streaming in *Elodea* leaf or other convenient material.

(a) Mount a leaf in water with a cover-slip and apply heat by irrigating with drops of warm water or by means of a hot-stage.

(b) Using a gas-chamber fixed to a slide with hard paraffin or Canada balsam, mount a leaf in a hanging drop on a large cover-slip so that the under side can be examined under the microscope. The upper edge of the chamber should be smeared with soft paraffin.

- (i.) Pass hydrogen from a gas supply stored over water, through the chamber. Streaming continues for over an hour in the absence of oxygen.
- (ii.) Pass washed carbon dioxide from a Kipp's apparatus through the chamber for fifteen to twenty minutes. Observe the result and then pass air through the chamber

for at least ten minutes, by displacement or by using a pump. Continue aeration until streaming starts again.

- (iii.) Put a few drops of chloroform in an otherwise empty wash-bottle and attach this to the gas-chamber so that the air entering is charged with chloroform vapour. Record your results systematically. Other possible materials are given in the Textbook, p. 355.

405. Mount an *Elodea* leaf upside down as before (Ex. 403) on an electrode slide, add a cover-slip; note the speed of protoplasmic streaming. (i.) Connect the electrodes direct to the terminals of a 4-volt dry-cell; observe the streaming during ten minutes. (ii.) Connect the electrodes to the terminals of an active induction coil and record the effect of the induced (tetanising) current.

Chloroplast Movements

406. Mount a filament of fresh *Mougeotia* in water with a cover-slip; note the position of the flat elongated chloroplast in each cell. Place the preparation in dull light for ten minutes; then determine quickly under the microscope the position of the chloroplast in several cells. Watch the chloroplasts as they slowly turn their edges towards the strong light used for the microscope.

407. (a) Expose some plants of *Funaria* or other moss or of *Lemna* spp. to sun, while similar plants are being kept in dull light, not darkness, for two to four hours. Compare the positions of the chloroplasts in representative leaves from the two sets.

(b) The same phenomenon as in Ex. 406 and (a) above may be found in slivers from the under sides of wood-sorrel leaves which have been subjected to sun and shade as above.

TAXISMS

Chemotaxis

408. **Bacteria.** Boil a few peas in 100 c.c. water; set aside for three days. Remove a drop of the turbid liquid from just below the surface; place the drop on a clean slide, and add a cover-slip. Note the bacteria more or less uniformly distributed. Set the preparation aside for half an hour; re-examine the distribution of the bacteria, noting particularly the zone around the margin of the cover-slip.

409. **Antherozoids.** Procure some fresh ripe fern prothalli. Prepare a few very fine capillary tubes about 0.1 mm. in diameter. Fill these with a solution of malic acid, 0.01 to 0.1 per cent. in tap water, by placing the tubes in a bottle containing the solution and attaching the bottle to a suction pump. Wash the outside of the tubes in tap water.

Mount the prothalli under a cover-slip in tap water, or rain water if available. Observe the liberated antherozoids, and then insert two or three capillary tubes of malic acid close together under the cover-slip at the *same* side of the preparation. Note the subsequent movements of the antherozoids.

Osmotaxis

410. Using bacteria as in Ex. 408, and fine capillary tubes as in Ex. 409, with solutions of potassium nitrate 0.02 M, 0.2 M, 1.0 M and 2.0 M, investigate the balance between chemotaxis and osmotaxis in relation to these organisms.

Phototaxis

411. Place a flat-sided open museum jar, containing active specimens of *Chlamydomonas* or other motile algal cells, in a box of about the same size, so that three sides and the top of the jar are well shaded. Set this arrangement in a dark corner. When inspection shows a green layer on the moderately lighted side of the jar, remove the jar and turn the green side to the back; repeat the exposure to dull light. This demonstrates *positive phototaxis*. Change the position so that the exposed side of the jar is illuminated by direct sunshine. The results should demonstrate *negative phototaxis* under the stimulus of strong light.

412. Many interesting variations of the experiments in Ex. 411 can be obtained with a long test-tube half-filled with water containing motile green algal cells. The tube is corked; laid horizontal; 5 cm. at one end are covered with a detachable tubular cap of black paper; the other end of the tube is exposed to bright light, while the middle portion is shaded and then other arrangements of light, shade and dark are made. The movement away from strong light may carry the organisms through the zone of medium light into darkness, from which they presently emerge and finally congregate in the medium zone.

Rheotaxis

413. Using the fresh active plasmodium of a myxomycete as the organism, arrange a beaker full of water on a glass plate with a strip of pure filter paper dipping to the bottom of the beaker, and hanging over the edge on to the glass plate. Place the plasmodium on that part of the wet paper which is on the plate, and set aside in a quiet place. Observe at daily intervals for a week, adding water in the beaker if the movement of the plasmodium is slow enough to make that necessary.

TROPISMS

GEOTROPISM

414. Position of Curvature

(a) **Root.** Mark a straight root, *e.g.* of broad bean which is about 5 cm. long, with equidistant marks, using, if possible, the opisometer with a very light touch. See Ex. 261, and

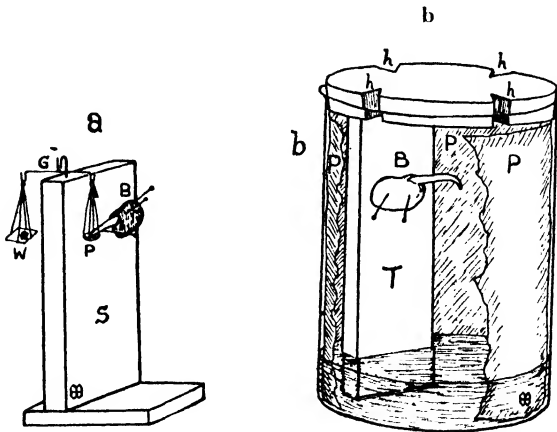


FIG. 29.—(a) S, a small wooden stand ; G, a wire beam balance with *w.* scale-pan for weights and P scale-pan with the root from B the bean touching the far side. The whole is small enough to be placed in a beaker like that in (b). (b) Glass beaker arranged as a moist chamber with sheet-cork cover and support T for seedling B ; PP, wet blotting-paper partly removed to show interior ; hh, air-holes in cover. The support T is removed when the apparatus (a) is inserted. A glass cover may be used if the beaker is kept in a dark room.

do not use a seedling which shows any sign of Sach's curvature. Set the seedling aside in a damp chamber such as a covered beaker lined with wet blotting paper (Fig. 29B), with the marked root horizontal. After two days note the relation of the region of curvature to the region of greatest growth.

(b) **Stem.** Treat a sunflower hypocotyl about 8 cm. long in the same way. The upward curvature may occur just under the cotyledons, or it may be spread along the whole

length, or it may occur just at the base of the hypocotyl. Sunflower seedlings vary.

415. Geotropic Pressure. (a) Fix a seedling so that a straight root lies horizontally in a little water over the surface of a layer of mercury at least 1 cm. deep. Observe the positive pressure developed during the geotropic curving of the root. If a wide-mouthed bottle with an air-hole in the cork be used, the seedling shows interesting developments during a period of two or three weeks.

(b) Prepare an inverted T arrangement with soft wood for the upright, S. Insert on the top a twice-bent pin and fit a wire beam balance with two micro-pans¹ as shown (Fig. 29A). Arrange a bean with a straight root horizontally with two pins, so that the edge of the concave pan just touches the root about 3 mm. from the tip; put a weight such as 0.10 or 0.05 gramme² on the flat pan. A pin may be used under the root near the scale-pan as an extra support. Set the apparatus aside in a moist chamber for twenty-four hours; note, and write an account of the result.

These experiments demonstrate that the downward curvature of the root is not due to a direct effect of gravity.

416. Plagiogeotropism

(a) Select a seedling of broad bean or pea which is just beginning to show secondary rootlets. Fix the cotyledons securely to a piece of sheet cork covering a beaker lined with wet blotting-paper (see Fig. 29 (b)). Grow the seedling in darkness until the longest secondary rootlets are 2 to 4 cm. in length. Draw a diagram showing the main root and the angles of the lateral rootlets. Return the beaker to its original dark position and leave it tilted at an angle of 45° for two days. Re-examine and draw another diagram showing the new positions of root and rootlets.

(b) If a wide-mouthed bottle be used as a damp chamber,

¹ The flat scale-pan is a cover-slip; the concave scale-pan is a piece of a small thin glass bulb, blown for the purpose; each is supported by two very thin wire filaments, crossed and fixed with Canada balsam on the bottom of each pan. The beam is one strand from thin brass picture wire; 6 cm. are straightened, turned in the middle once round a pin, and bent down then up again to form end hooks for the rings of the pan supports. The beam is carefully balanced with a bent wire rider of the same material, hung on one of the hooks and cut gradually until the beam is level.

² 0.2 gm. is too much; 0.1 gm. may prevent the downward curving; 0.05 gm. allows curvature to take place.

any experimental angle up to complete inversion of the seedling can be obtained ; and this is a more convenient arrangement for first-year work.

417. Flowers. (a) Select a young inflorescence of monkshood, foxglove or other raceme of zygomorphic flowers, with the upper flowers still in bud ; insert the cut end through a cork or rubber stopper ; fix the stopper in a 20-cm. length of suitable glass tubing ; invert and fill the tube with water. The latter part of the manipulation should be carried out quickly ; when the stem has been sealed through the stopper a fresh end should be made by cutting about 1 cm. off under water, then the tube with water should be affixed as quickly as possible. Leave the inflorescence, inverted and supplied with water, for about a week, taking notes and sketches of the buds as they are orientated by gravity stimulus in unusual relations to the axis (see Fig. T 856).

(b) Repeat the above experiment with young spikes of *Orchis*.

(c) Modify the above experiment, using a closed tube for water supply and growing the inflorescence in a horizontal position on a constantly rotating klinostat for a similar period of time.

(d) Arrange a young daffodil scape firmly in a tube of water ; record the angle between the stem and the flower. Tilt the tube 45° from the vertical and record the angle again after twenty-four hours.

N.B. Light is required for the initiation of this response to gravity.

418. Fix the flat seeds of *Cucurbita* firmly in moist soil, so that some seeds lie flat and others have their flat side vertical. After two weeks examine the orientation of the "peg." (see Fig. T 858).

419. (a) Mark a well-grown, vertical **grass node** with two

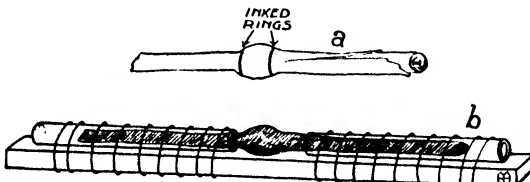


FIG. 30 (a) see Ex. 419 (a) ; (b) see Ex. 419 (b).

circles at a measured distance, *i.e.* two circles 4 mm. apart ; bury the haulm in a horizontal position in loose moist soil

or coconut fibre (see Fig. 30 (a)). After two days re-examine the specimen and again measure the distance between the circles, especially on the upper and under sides. Draw an annotated diagram of your results.

(b) Fit a similar grass node with two glass tubes and bind the tubes to a narrow slip of wood (see Fig. 30 (b)). Bury as in (a) and leave for a week. Examine and make an illustrated record of the resulting specimen.

Klinostat Experiments

420. Stem. Place a healthy, *securely potted* plant on the klinostat in a horizontal position. Leave in constant

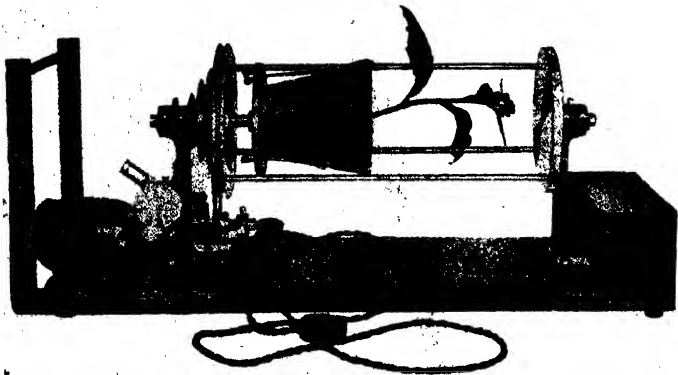


FIG. 31.—Small's Improved Klinostat. Electrically driven, this gives rotation periods of ten to thirty minutes, in the horizontal or vertical position.

rotation for twenty-four hours. Stop the klinostat for one hour; mark the uppermost part of the pot; and leave again in constant rotation until a visible curvature appears. Note (i.) the direction of the curvature in the stem, and (ii.) the time taken for that curvature to develop.

421. Root. Arrange a seedling with a straight root vertically in a damp chamber (see Fig. 29 (b)); attach this securely to a vertical klinostat. Turn the apparatus into a horizontal position, and rotate for twenty-four hours. Stop the klinostat for an hour; mark and resume rotation as

before. Note (i.) the direction of curvature and (ii.) the time taken for the first appearance of the curvature.

422. Presentation Time. By means of a graded series of "stop periods" determine the minimum period, of continuous stimulation, for geotropic curvature in (a) a stem, and (b) a root. The "stop period" should gradually increase; a useful series is 10, 12, 14, 16, 18, 20, 25, 30 minutes; and rotation between the "stop periods" should last for at least ten times the preceding period of stimulation.

423. Relaxation Time. (a) Having determined approximately the presentation time for the stem or root of one plant, investigate the summation of successive stimulations by using successive stop or stimulation periods of one-fifth presentation time, and rotation periods or rest periods of only three or four times the length of the "stop period." From the number of stop periods required for curvature, calculate the relaxation time approximately thus: Let PT be presentation time, S stimulation or stop time, R rest or rotation time, all in minutes, and N the number of S periods required to get response. Bring NS up to next higher multiple of PT , as $NS + z$ then $\frac{PT}{NS + z} = y$, the fraction of full stimulation which has been summated to get response. If the value of y is from $1/2$ to $1/10$, and the fraction of relaxation time used is x , then $xy = \frac{1}{20}$ or $x = \frac{1}{20y}$, also $\frac{1}{x}R = rt$, the relaxation time in minutes for S minutes of stimulation. If rT be relaxation time as a ratio, then $\frac{rt}{S} = \frac{rT}{1}$.

Example. $PT = 10$, $S = 2$, $R = 6$, $N = 9$, then $NS = 18$, $z = 2$, $NS + z = 20$, $y = 1/2$, then $x = 1/10$, $rt = 10 \times 6 = 60$ for 2 mins., and $rT : 1 = 30 : 1$.

N.B. Relaxation time is a ratio, not a definite period. The actual time in minutes depends upon the duration of the stimulation or "stop-periods," as well as upon the plant used.

(b) The calculated value of Relaxation Time may be checked by experimental approximations, using the longer members of a series of periods first, but this takes up too much time to be an ordinary student's experiment.

424. Reaction Time. Set up a seedling with its main root vertical and marked near the tip with a very small spot of Indian ink. The seedling should be firmly fixed within a museum jar with flat sides arranged as a moist chamber (see Fig. 296), and the marked side of the root should be near but not touching the glass of one side, which is left clear of paper. Prepare the horizontal microscope, and lay the jar on its side so that the root is horizontal.

¹ The exact equation may vary slightly, and this is an average value for approximations only.

Observe carefully and record the positions of the ink spot at five-minute intervals during the following thirty to sixty minutes. Determine from these observations the reaction time of the root used.

425. Perception Time. This may be determined approximately by electrical methods, but is too short to be determined in class, except by more advanced students, who will find a suitable apparatus and method described by the present writer in *Proc. Roy. Soc. B.*, 90, p. 349, 1918.

426. Rectipetality

(a) Modify Ex. 417 (d) with a daffodil bud already bent, by fixing the tube on a horizontal klinostat and rotating continuously for twenty-four to forty-eight hours. Record the angle between flower and stem before and after rotation.

(b) Modify Ex. 420, using mustard or pea seedlings, by continuing the rotation on a horizontal klinostat for twenty-four to forty-eight hours after curvature has been found.

The geotropic curvatures in both (a) and (b) may be found to disappear; this straightening effect is known as *rectipetality*.

427. Centrifugal Force. Attach a series of straight seedlings firmly by two pins to the arms of a Knight's Wheel which is moving at an appropriate speed, *i.e.* from one to five revolutions per second; arrange a water supply and keep the wheel in constant rotation for forty-eight hours. The seedlings should be fixed at various measured distances from the centre of the wheel, so that they are subjected to centrifugal force of various strengths. After forty-eight hours examine and draw the seedlings to show the new positions of the root and stems.

The strength of the centrifugal force varies directly as the distance from the centre of the Knight's Wheel, and directly as the square of the speed in revolutions.¹ A speed of 100 revolutions per minute, with arms 15 cm. long, gives a graded series of departures from the vertical position; and with various speeds the relation of gravity to centrifugal force can be studied.

The apparatus illustrated (Fig. 32) has been designed to give a slow revolution, the speed of which can be calculated from that

¹ The formula is $F = 4 \pi^2 m N^2 r$. For comparison with the effect of gravity on the same material m is constant; $4\pi^2 = 39.4$; N , the angular velocity in revs. per second, with 20 revs. per minute is .33 and $N^2 = 0.1089$; r is the radius in centimetres. The ratio 7.5 to 1 of the driving wheel and jigwheel gives a practical increase of N up to 2.33 and of N^2 up to 5.4. With $N = 2.0$ and $r = 6$ cm., $F = 945.6$ or *ca.* 1 g; and other values of F are obtained along the rotating arms, *e.g.* 0.5 g at 3 cm., 1.5 g at 9 cm. up to 2.5 g at 15 cm. If the jigwheel is to be adjusted it should be borne in mind that F varies as the square of the speed. Values up to about five times g can be obtained at 15 cm. with a 7 to 1 ratio and an increase in the speed of the electric motor.

of the driving pulley of the klinostat ; the speed of the arms can be decreased by moving the horizontal tyred wheel up or down towards or away from the centre of the vertical driving wheel which connects with the first stage of reduction in the klinostat. When the driving pulley is at 0.2 revs. per min. the driving wheel

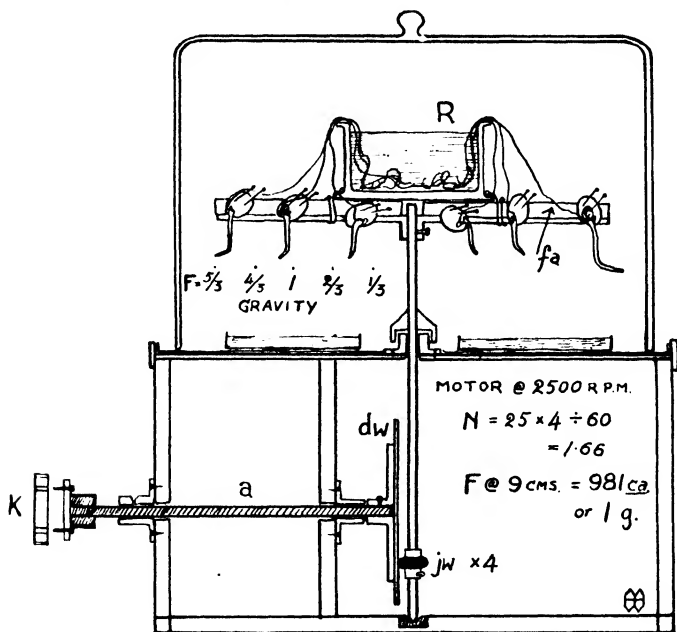


FIG. 32.— Knight's Wheel for use with Small's Klinostat : *a*. driving axis attached to Klinostat at *K* *dw*. driving wheel ; *jw*. jigsaw wheel or tyred wheel ; *fa*. four-armed rotating " Knight's Wheel " of brass and thick cork to the sides of which seedlings are pinned as shown ; *R*. water reservoir with cord wicks.

is at 20 revs. per min., so that with the tyred wheel at the circumference of the driving wheel the speed of the arms is 150 revs. per min. ; by moving the tyre up half the radius of the driving wheel the speed is reduced to 75 revs. per min., and so on, the speed of the tyred wheel being proportional to its distance from the axis of the driving wheel.

Perceptive Region

428. (a) **Czapek's Boots.** Prepare three or four short light "Czapek's boots," by bending closed glass tubes

about 2 mm. from the closed ends and cutting off the right-angle bends (see Fig. T 861). Attach these to sheet-cork by means of melted hard paraffin and arrange suitable seedlings in a moist chamber so that the roots will grow into the boots. When the root-tips have been fitted, detach the glass boots carefully from the paraffin; and fix the roots in various positions with relation to the vertical. Make drawings to show the initial positions of the roots and boots. Return the sheet-cork with the seedlings to the damp chamber and set the apparatus aside in the dark. Examine at daily intervals until the roots are all growing horizontally; then add to your previous drawings the new positions of roots and boots. State briefly the significance of your results.

(b) Using seedlings of *Setaria* or *Sorghum*, fix the tips of two coleoptiles in closely fitting glass tubes. Support the tubes horizontally and keep the seedlings thus arranged in a moist, dark chamber. Observe and make illustrated records of the resulting curvatures at daily intervals for two or three days. Write an explanation of these results, including their significance in relation to the perceptive region and the continuance of gravity stimulus.

429. Decapitation. Examine a root-tip of broad bean carefully, using a pocket-lens *against* a strong light. You should be able to see a V- or U-shaped translucent zone surrounding the dense greyish rounded meristematic region. In the following experiments you are required to hold the root vertically upside down and to cut off as much as possible of the translucent tip and as little as possible of the denser part. This operation separates the statolith region from the meristem. A very sharp razor should be used with great care. Seedlings of broad bean with roots about 5 cm. long should be used, with a moist chamber for subsequent developments.

(a) Remove the root-cap by an oblique cut, (i.) at right angles to the main plane of the cotyledons, and (ii.) in the same plane as the cotyledons.

(b) Remove the root-cap by a straight cut exactly at right angles with the axis of the root.

(c) Lay a seedling with its root horizontal for fifteen minutes; then remove the root-cap as in (b).

(d) Remove the root-caps from two roots and by means of a camel-hair brush moistened with saliva replace the root-caps so that they are interchanged, *i.e.* the cap of the first is replaced on the root of the second seedling and the cap of the second on the root of the first.

Each seedling, whenever it has been operated upon, should be

fixed with its root horizontal in the damp chamber. Set all four experiments aside in the dark and examine daily during a week. Make an illustrated record of the final results, and write a brief account of their significance.

430. Oxygen and Geotropism. (a) Modify Ex. 414a by submerging the whole seedling in previously boiled and cooled water, with the root horizontal. The equidistant marks may indicate a certain amount of growth; and this, together with the effect on geotropic response, should be recorded.

(b) Arrange a damp chamber so that hydrogen from a reservoir or from a generator can be passed through. Subject two or three seedlings, with marked roots horizontally placed, to an atmosphere of hydrogen and compare the results with those of the previous experiment.

431. Statoliths. Investigate the movable starch in certain plant cells, using the iodine test for starch, recording the number of grains in each of several cells and taking an illustrated record of the position of the grains (see Fig. T 862).

(a) Cut longitudinal sections of root-tips from roots which have been kept (i.) vertical, and (ii.) horizontal for fifteen minutes before being cut. Record as directed.

(b) Cut a narrow longitudinal V in a stem, *e.g.* of sunflower or clover or bean; cut the stem into three pieces each with the V cut present; keep one piece vertical, a second piece horizontal with the V up and a third with the V down. After twenty minutes cut transverse sections of each piece and note the position of the starch grains of the endodermis in relation to the V cut which gives a point for orientation. Longitudinal sections of the erect stem should also be examined. Record as directed.

432. Hormones. (a) Take a 10-cm. length of fresh young bean stem and split one end in half longitudinally; note the direction of curvature due to differential turgidity (see Fig. T 863).

(b) Take another 10-cm. length of fresh young bean stem; lay it horizontally on a clean glass plate under a bell jar lined with wet paper; after fifteen minutes cut the stem carefully, with a clean, sharp razor, in half without raising it from the glass plate. Lay the upper half with its cut surface next the glass and leave the lower half with the cut surface upwards. Observe at intervals during two hours,

and record the final curvatures found after twenty-four hours. Write a brief account of the significance of your results.

Orientation of Branches and Leaves

433. The arrangements of branches and of leaves may be conveniently taken at this stage, although these are due in varying degrees to influences other than plagiogeotropism, e.g. heliotropism, hyponasty, epinasty, etc.

(a) In the spring fix a young branch of a suitable shrub or tree so that its developing apex is already in the position which it would assume later. If the apex develops at its normal angle with the vertical, the usual movement is shown to be either plagiogeotropic or plagioheliotropic; hyponasty is eliminated. Suitable plants are lime, elm and hazel.

(b) Examine the internodes and petioles on side branches of *Philadelphus*, *Buxus* or *Lonicera* spp., or other shrubs with leaves which are opposite and decussate in origin, but arranged on the side branches in one plane when mature.

(c) Examine the various twists by which the leaf blades on side-branches of the yew (*Taxus*) are arranged in one plane (see "P.-l. P. I." No. 182).

PHOTOTROPISM

Positive Phototropism

434. Place a pot of vertical mustard seedlings which have been grown in full light in a phototropism box, i.e. a box with only a slit at one side for the admission of light. Observe and record the appearance of the seedlings twenty-four hours later. This experiment demonstrates very distinctly the same positive phototropism as is shown in lesser degree by potted plants near house windows.

435. Arrange two similar pots of mustard seedlings one exposed to lateral illumination from only one window, and the other beside the first, but rotating on the vertical klinostat. Compare and record twenty-four hours later.

436. Using a phototropism box with coloured glass slips, investigate the response of mustard or other seedlings to light of different wave-lengths. Record your results, with a brief account of their significance.

437. **Perceptive Region.** (a) Grow at least a dozen *Setaria* or *Sorghum* seedlings in complete darkness until the coleoptiles are

1.0 to 1.5 cm. long. Prepare six small tin-foil caps about 3 mm. long to fit over the tips of the coleoptiles. Fit the caps on six seedlings and leave the others free. Place the pot of seedlings in a phototropism box, and record the result after twenty-four hours.

(b) Place loosely fitting tin-foil caps on the tips of mustard, cress, sunflower or maize seedlings, leaving others in the same pot free. Expose to unilateral illumination as before and record the result after twenty-four hours.

Compare and write a brief account of the significance of the two sets of results from (a) and (b); comparing also the normal stem in its response to gravity.

Negative Phototropism

438. Grow some mustard seedlings in loose coconut fibre until the roots are 2 to 5 cm. long. Remove the seedlings; wash the roots gently in water, and pin the cotyledons to a strip of sheet-cork fixed across a beaker. Add sufficient water to immerse the roots, and expose the seedlings to unilateral illumination in a phototropism box. Record the appearance of the seedlings including both roots and stems after twenty-four hours.

Plagiophototropism

439. In order to separate clearly the effects of gravity and of light upon the plagiotropic orientation of leaves, it is necessary to rotate the experimental plants on a horizontal klinostat in such a way that the gravity stimulus is eliminated or neutralised while, at the same time, the plant is exposed to light always falling upon the same side of the plant.

Fix a 5-inch flower-pot on a klinostat; prepare a cover of black paper for the pot with a slit 2 cm. wide and 10 cm. long. Tie a ball of wet cotton-wool firmly around the base of the experimental plant and wedge the cotton-wool into the flower-pot with three strips of wood, so that the axis of the plant is at right angles to the rotating axis of the klinostat. Tie the black paper cap on the pot; place the klinostat so that the slit in the cap faces a window light, and rotate the plant continuously for forty-eight hours.

Any rearrangement of the leaves so that they receive better illumination by torsion of the petioles or leaf-bases is due to plagiophototropism. Suitable materials are short leafy twigs in the young stages of privet, Portugal laurel, *Veronica salicifolia*, etc., short leafy shoots of deadnettle, wood sage, *Mimulus* and *Lysimachia*, or rooted plants of lesser celandine, ground ivy, etc. Growth curves, hyponastic or epinastic, may be disturbing factors, especially in the herbaceous materials.

HAPTOTROPISM

440. Using a waterproof adhesive, such as shellac mixed with a very small quantity of methylated spirit to form a sticky varnish, fix a millimetre square of rough cardboard to one side of the tip of a bean root. Set the seedling to grow in a moist chamber, and record the results daily for about a week.

441. Prepare a glass beaker two-thirds filled with rather large pieces of broken flower-pot material; add a layer of wet coconut fibre and a top layer of soil. Wet the whole so that there is at least 1 inch of free water in the bottom of the beaker. Select a few seedlings with radicles about 4 cm. long, and plant these in the prepared layer of soil, so that the radicles can be seen from the outside, amongst the earthenware. Set the beaker aside for a week, and then study the result carefully. Reconstruct for yourself the course taken by the root-tips and write an illustrated account of the phenomena from the point of view of haptotropic movements.

442. Perceptive Regions. Examine, under H.P., fresh preparations of the sensitive filaments of *Lapsana communis*, *Carduus crispus*, *Cnicus lanceolatus*, or *Centaurea cyanus*; also the inner surface of filaments of *Berberis* or *Mahonia*. Note and draw the tactile pits as the haptotropic perceptive regions.

443. Pulvini. As the motor regions in many haptotropic movements, the cushions or pulvini of various plants should be examined.

(a) Cut transverse and longitudinal sections of the elongated pulvinus of *Phaseolus*. Make line diagrams of both views and draw a small part of the loose tissue with intercellular spaces under H.P.

(b) Using plants of *Oxalis acetosella* potted, or preferably in their natural environment on a warm day, stimulate the three pulvini of a leaf one at a time by rubbing the under surface of the pulvinus gently with the end of a thin twig or pencil point.

Note (i.) the downward movement of each leaflet; (ii.) the absence of any transmission of the stimulus; (iii.) under a good lens, the appearance of wrinkles on the under side, while those previously present on the upper side disappear (see "Pocket-lens Plant Lore," No. 75). If time is available, observe the recovery to the original position. This takes

about an hour, and the wrinkles may then be seen to be again distributed as they are in undisturbed leaves with the leaflets expanded.

444. (a) Revise the experiments on haptotropic movements of tendrils, styles, and stamens, also of *Drosera* and other insectivorous plants, Chapters XVII, XXI, and also XII.

(b) As part of this revision, the more advanced student should attempt to measure approximately the rates of growth of a stimulated tendril and an unstimulated tendril. This may be done without touching the tendrils, by making as accurate drawings as possible of two tendrils of *Lathyrus odoratus* and measuring the drawings. It is convenient to tie a selected leaf to a support so that the tendrils are not stimulated by accident; one drawing is made of two tendrils; one of the two tendrils is stimulated and about an hour later a second drawing is made of both tendrils. The difference is obvious, but measurement of the drawings gives a roughly quantitative comparison.

445. Mimosa. The sensitive plant *Mimosa pudica* responds to a variety of stimuli. The complete range of movement is somewhat complicated. This should be observed and a record made of the movements of the pinnules of the main divisions of the palmate leaf and of the petiole.

(a) **Haptotropism.** Touch a pinnule gently on the under side of its pulvinus and note the local response. Observe the lack of response to a similar touch on the upper side of the pulvinus. Strike another pinnule sharply and note the transmission of the stimulus to other pinnules.

(b) **Thermotropism.** Bring a hot needle carefully near a pinnule without touching the leaf at all. Note the response. Bring a small flame, e.g. from a match, below the terminal pinnules of one division. Note the transmission of the stronger stimulus.

(c) **Chemotropism.** Place one drop of water on one pinnule, one drop of 0.880 ammonia on another pinnule, and one drop of concentrated hydrochloric acid on a third pinnule. Each pinnule should belong to a different division of the same leaf. Observe and record the responses and the way in which the stimulus travels.

(d) **Electrotropism.** Wind a bare wire gently round the stem of a *Mimosa* plant; connect this to one terminal of an active induction coil. Connect another wire to the other terminal; attach a piece of soft fine wire to the end of the second wire and touch the upper surface of a leaf pulvinus with this fine wire.

Compare the response in this case with the absence of haptotropic response to a simple touch on this part, see above (a). Check the absence of haptotropism by touching a pulvinus in a similar way with the same wire detached from the induction coil.

HYDROTROPISM

446. (a) Grow some mustard or other small seedlings in moist coconut fibre laid upon a shallow tray of perforated zinc or wire gauze over water. When the roots begin to emerge, turn the tray at an angle of 45° to the vertical and keep the fibre well moistened. Make an illustrated record of the result after two days.

(b) An alternative method of demonstrating positive hydrotropism is the following. Arrange a series of seedlings with short roots through the holes of a porous "hydrotropism funnel." Fill the interior of the funnel with dry sawdust or coconut fibre to keep the seedlings in position; place the stem of the funnel in a "hydrotropism vase" full of water. Observe and record the results as before.

THERMOTROPISM

447. This is best studied by means of a specially made Thermotropism Box, as illustrated in Fig. 33. The wide mesh wire gauze *WG* is placed in position in the slots of the wooden ends *EE*.

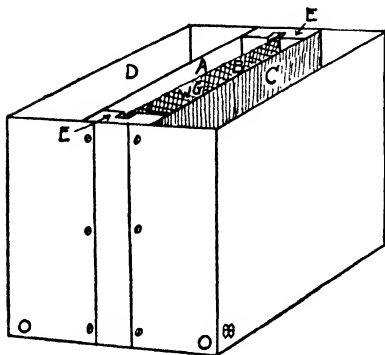


FIG. 33.—Thermotropism Box (for explanation see text).

(a) The compartments A and B are then filled with loose, moist coconut fibre up to the top of the gauze. Twenty mustard seeds are then sown above compartment B in the top layer. After five or six days in which normal germination takes place, the metal compartment C is kept filled with ice for three days, the compartment D is then filled with ice, and C is emptied; OO are drainage openings (Fig. 33). After

another three days the alternation of cold in C and then in D may be repeated. The wire gauze and fibre should then be removed carefully, when a considerable number of roots will be

found to have passed first from B to A through the gauze and then back again from A to B.

(b) The same alternate application of hot water, especially if it is applied to cotton-wool packed in the compartments C and D and renewed about three times a day, gives the same effect of interwoven roots.

Chemotropism

448. (a) Revise Exs. 222, 223, 226, which are examples of chemotropic stimulation.

(b) See also Ex. 445 (c) above, as another example.

(c) Revise Ex. 361 (pollen tube), which is an example of chemotropism with much biological significance.

Traumatotropism

449. Revise Ex. 429 (a), which is a special case of traumatic stimulation and response.

450. Since the result of oblique decapitation of the root-tip may be attributed to greater growth with a greater amount of meristem left on one side, the student should take a very sharp razor and cut a small notch from the cortical cover of a bean root-tip; place the seedling in a moist, dark chamber and observe the resulting traumotropic curvature.

Nutation

451. Revise Ex. 294, which deals with the comparatively large and comparatively regular spiral movement of the apex of a twining stem.

452. The smaller and less regular nutatory movements of ordinary stem apices can be followed by a suitable method of magnifying the linear dimensions of the movements.

(a) Fix a small drop of red sealing wax not more than 1 mm. diameter to the stem apex of a young potted plant, *e.g.* sunflower or bean or pea. Arrange the pot on the floor in a box about 9 in. high and 6 in. square. Fix two white threads, crossing each other at right angles, on a retort ring, if necessary, 15 to 20 cm. above the stem apex, arranging the cross of the threads so that it is directly above the sealing wax. Arrange a glass plate on a clamp-stand about 50 cm. above the crossed threads. Mark a dot with Indian ink on the glass plate, so that this dot, the cross of the threads and the drop of sealing wax are in alignment. Repeat the marking of aligned dots at intervals of thirty to sixty

minutes, as convenient during the next twenty-four hours, numbering the dots as they are made. Transfer the record of numbered dots to your notebook,¹ and connect up the dots in proper sequence. Note that this method gives a *reversed* record of the actual movements of the stem apex.

(b) An alternative method of magnifying these nutatory movements and obtaining a *direct* record may be found in "Power of Movement in Plants," by C. Darwin (p. 7), which is a book that all students should read at this stage.

Epinasty

453. Select a plant of lesser celandine which has its leaf-blades upon the ground; uproot the plant carefully, digging up only the centre of the earth over which the shoot is spread. Note the strong downward curvature of the petioles, which is due to greater growth turgidity on their upper sides.

454. Investigate in the same way other similar plants with radical leaves bent towards the surface of the earth, e.g. *Plantago* spp., *Potentilla anserina*, sundew, butterwort, etc.

455. (a) Observe and make illustrated records of epinastic growth, (i.) in the uncoiling of a young fern frond, e.g. *Aspidium*, and (ii.) in the opening and final downward curving of the lateral branches of *Equisetum arvense*.

(b) Epinastic effects as such may be distinguished from plagio-geotropic effects by keeping the above plants either rotating on a horizontal klinostat, while the movement is observed, or by keeping securely potted plants upside down, with a screen around the soil, in the dark for several days. The *Equisetum* main axis may turn upwards, but the lower lateral branches still show an epinastic curvature. Geotropic effects complicate the case of the fern frond and also that of *Rumex* leaves.

Nyctitropism

The natural periodic movements of leaves and flowers whereby they assume different aspects with the alternation of day and night are frequently temperature effects, but in the case of clover (Ex. 456 below) light is the dominant factor.

456. **Light.** (a) On a warm, sunny day, place a 5-inch flower-pot, with a cork in the hole at the bottom, in the

¹ This may be done easily by using the glass plate on a copying table with an electric lamp below. The dots then show through the paper.

sunshine for two hours, in order to heat it. Then place the heated pot over some clover shoots. Two hours later compare the clover leaves which have been in warm darkness with some which have been for at least the same period in warm outdoor shade. Make an illustrated record of your results, including a careful drawing of at least one representative leaf of each kind.

(b) Repeat this experiment with the open flower-heads of the daisy (*Bellis perennis*). Note carefully the angle of the ray florets and measure the distance between the tips of the florets diametrically across the capitulum.

457. Temperature. (a) Attach by means of sticky shellac varnish¹ two slender glass filaments about 15 cm. long vertically to opposite perianth segments of a closed tulip flower. Set up a millimetre scale so that the distance between the upper ends of the filaments can be readily measured. Fix a thermometer close to the flower. Read the temperature and measure the distance. Then bring by stages an electric hot-plate nearer to the flower, repeating your observations of temperature and degree of opening of the perianth at each successive stage, and allowing ten minutes for equilibrium to be reached after each approach of the hot-plate. After two definite stages of opening have been obtained, withdraw the hot-plate and record the subsequent closing movements with fall of temperature.

(b) Repeat the above experiment in a dark room using a daisy flower-head, which has been partially closed by being kept in the dark. Compare Ex. 456 (b); both light and temperature have an effect upon the daisy.

458. Observe, as opportunities arise, the day and night positions of the leaflets of *Oxalis* spp., and *Marsilea*; the leaves of *Nicotiana* (Fig. T 875), *Mimosa*; the cotyledons of cabbage and radish, etc.

459. Autonomous Movements. *Desmodium* and *Averrhoa*, the classical examples, are not always readily available, but slower and slighter movements can be detected in clover leaflets. Using a plant of clover which has been potted for at least twenty-four hours, fix the petiole of one leaf firmly to a small stick stuck into the soil. The petiole should be bound to the stick, by means of gummed paper, in such a way that the terminal leaflet is quite free to move in any direction. In order to follow the oscillations of the leaflets more clearly a 4 cm. square of white cardboard

¹ See Appendix II.

should be marked with two black lines crossing in the centre at right angles, and this should be fixed by means of a clamp-stand or another stick about 2 cm. behind the leaflet, with the cross of the lines level with the pulvinus. Observations of the exact position of the leaflet in relation to the lines should be recorded at hourly intervals throughout a period of at least four hours.

CHAPTER XXVII

HEREDITY

THE most useful approach to practical work in heredity, for the first- or second- year student, consists of the solution of theoretical problems. The actual work of selection, crossing and subsequent segregation is too lengthy a process for a two-year course ; and an intelligent appreciation of the results obtained by others is the most that can be expected from beginners.

The problems set below are intended to illustrate only such phenomena as are considered in the Textbook.

460. Given pure breeding strains of tall and dwarf peas respectively, what would be the result *in the plants of the following year*, of :—

- (a) Self-pollinating flowers of each strain ;
- (b) Cross-pollinating flowers of the two strains ?

What can you deduce from this concerning the relation of the characters, tallness and dwarfness ?

461. Give a brief account of how you would make sure of self- and cross-pollination in the above experiment.

462. What would be the result of self-pollinating flowers on the F₁ plants obtained from Ex. 460 (b) ?

463. (a) Given that two strains, A and B, are crossed, and that the F₁ generation when selfed yields progeny resembling the parents as regards one pair of opposed characters in the ratio of 3B : 1A, what deductions could you make concerning the characters studied ?

(b) The **phenotype** is the organism as seen ; the **genotype** is the organism as it is shown to be by genetic experiments, or its "factorial constitution." How many phenotypes and how many genotypes would occur in the F₂ generation as the result of crossing A and B and selfing the F₁ progeny ?

(c) What would be the constitution of each genotype ?

(d) How is this related to the appearance of the phenotype and to the relation of the characters considered ?

464. Given two strains of peas which breed true for the

tall-dwarf pair of characters, what is the minimum number of years in which the behaviour of these characters in inheritance may be fully tested ?

465. Given two strains of peas which breed true for the yellow-green or round-wrinkled pair of seed characters, what is the minimum number of years in which the Mendelian inheritance can be fully tested ? Yellow is dominant over green and round over wrinkled.

466. Given a mixed population of peas, what is the minimum period of time in which you could obtain strains breeding true for : (i.) dwarfness, (ii.) tallness, (iii.) green seed, (iv.) yellow seed, (v.) round seed, (vi.) wrinkled seed ? Write, briefly and clearly, the reasons for the differences in the various periods.

467. Draw diagrams to show the phenotypes and genotypes which would result from " back-crossing " the F₁ generation of Ex. 463, with (i.) the pure or homozygous dominant, and (ii.) the homozygous recessive. How would the " back-crossing " be carried out with an annual plant such as the pea ?

468. The plants resulting from a cross between two strains of peas yield yellow and green seeds in equal numbers ; what was the genetic constitution of the parents with regard to the factors for these characters ?

469. Using the facts given in Ex. 465, consider the case of a pure strain of pea with yellow round seeds crossed with another pure strain of pea with green wrinkled seeds, the crossing being followed by self-pollination of the F₁ hybrid.

(a) How many different kinds of gametes are available in the F₁ generation ?

(b) How many phenotypes and how many genotypes would occur in the F₂ generation ?

(c) What would be the ratio of (i.) the different phenotypes, and (ii.) the different genotypes ?

470. Extend the above consideration to the following cases, analysing the F₁ and F₂ results for :

(a) Pure breeding yellow wrinkled $YYrr$ crossed with pure breeding green round $yyRR$:

(b) Heterozygous $Yyrr$ wrinkled crossed with green homozygous round :

(c) Heterozygous $YyRr$ " back-crossed " with the original parent strains, *e.g.* (i.) homozygous yellow round, and (ii.) with homozygous green wrinkled.

471. Given a set of three dominant characters, such as tallness, yellow and round seed together with a corresponding set of three recessive characters, such as dwarfness, green and wrinkled seed ; calculate for F₂ the genetic composition, numbers of phenotypes and genotypes, and the ratio of these when a pure dominant¹ is crossed with a pure recessive and the F₁ plants self-pollinated. How many different kinds of gametes are available for the production of (a) the F₁ generation, (b) the F₂ generation, and (c) the F₃ generation ?

472. When a white-flowered "four o'clock" (*Mirabilis jalapa*) is crossed with a red-flowered variety of the same species, all the F₁ flowers are pink and, when these are either self-pollinated or cross-pollinated amongst themselves, the resulting F₂ plants are of three kinds with red, pink, and white flowers respectively.

(a) Draw a diagram to show the gametes involved in the production of the F₂ generation, and its genotypic and phenotypic constitution.

(b) How many phenotypes and how many genotypes occur in F₂ ?

(c) In what ratio do these occur ?

(d) What is the relation of the characters red and white ?

473. One strain of wheat with red kernels is crossed with another strain having white kernels ; the F₁ generation has light red kernels ; compare the incomplete dominance in Ex. 472. The F₂ generation, from self-pollinated plants, shows a ratio of fifteen plants with reddish kernels to one plant with white kernels. The reddish character shows four degrees of depth in the red colour.

(a) Which Mendelian ratio is indicated ?

(b) Draw a diagram to show the gametes involved in the production of the F₂ generation, and its genotypic and phenotypic constitution. How many factors for "redness" are present, and what is the relation of these factors ?

474. Another strain of wheat with red kernels is crossed with a strain having white kernels ; the F₁ generation has light red kernels as above. The F₂ generation, from self-pollinated plants, is again of two kinds superficially, but the ratio is sixty-three reddish to one white.

(a) Which Mendelian ratio is indicated ?

(b) Draw a diagram to show the possible gametes and the resulting genotypes and phenotypes of the F₂ generation.

¹ *I.e.* pure dominant in respect to all the three pairs of characters considered.

(c) How many factors for "redness" are present and what is the relation of these factors ?

(d) How many degrees of redness should be present in the F₂ generation ?

(e) In what ratio should these different phenotypes occur ?

475. In the sweet pea, red flowers nearly always have erect standards, very seldom hooded standards, whereas purple flowers commonly have either erect or hooded standards.

(a) Taking *B* and *E* as the dominant blue¹ and erect factors, *b* and *e* as the recessive red and hooded factors draw a series of diagrams to show the various genetic arrangements in the following possible crosses: (i.) red erect with purple erect, (ii.) red erect with purple hooded.

(b) If redness and hooded standard never occur together, what conclusions can you draw from the above diagrams combined with this extra fact, (i.) as regards the possible kinds of gametes, (ii.) as regards the positions of the genes or factors for the various characters in the chromosomes concerned ?

476. In the sweet pea purple colour (*B*) and long pollen (*L*) are dominant over red colour (*b*) and round pollen (*l*). in an F₂ generation, grown from an F₁ lot produced by crossing a pure breeding purple long strain with a similar red round strain, four phenotypes occur, *i.e.* plants with purple² long, purple round, red long and red round characters.

(a) If the ratio in which these phenotypes occur is 177 purple long; 15 purple round; 15 red long; 49 red round, what is the ratio in which the gametes occur, *i.e.* *BL* : *Bl* : *bL* : *bl* ?

(b) If the gametes *Bl* and *bL* did not occur at all, would the case then be similar to that in Ex. 475 ?

(c) Assuming that the gametes *Bl* and *bL* occur in the ratio of 1 : 7 of either of the other types of gametes, how may the existence of the *Bl* and *bL* types be explained ?

(d) Since *BL* and *bl* occur in the ratio of 7 : 1 of the other kinds of gametes what conclusions can you draw as to the position of the genes or factors for the various characters in the chromosomes concerned ?

(e) What percentage of crossing-over is indicated, if the normal gametic output of the F₁ plants is taken as *BL* and *bl* ?

Note. The second edition of the Textbook requires revision of this particular phenomenon. The positions of *L* and *l* in Fig. 880 should be reversed and the text on pp. 383-384 altered to accord with the amended figure.

¹ Purple is given by the presence of both blue and red; red therefore occurs in the absence of the *blue* colour factor.

² See footnote to Ex. 475.

CHAPTER XXVIII

EVOLUTION

THE process of natural evolution is normally too slow, the available experimental examples are as yet too critical and the results too uncertain to be suitable for students' experiments. Ecogenesis (see Textbook, p. 388) may in time become a laboratory procedure. In the meantime the student can be on the outlook for examples of large or mutational changes; for example, one plant out of an extensive normal daisy population on the writer's lawn showed mutation in 1930 from the normal full-green leaf to a variegated leaf with broad, pale yellow lines over the main veins. This plant is still (1931) propagating vegetatively more or less in the normal way. Examples of bud mutations such as reversion of the cut-leaved beech to the normal form on some twigs of the tree, and also reversion of normal spineless pear-tree branches to the spiny form of the ancestral wild pear, may be observed occasionally; and serve as examples of sudden large changes in apparently stable organisms.

In addition to occasional observations of such mutational or discontinuous variations, the student should study carefully at least one case of normal or continuous variation, in order to be able to appreciate the critical analysis of these phenomena which is necessary for the scientific demonstration of the processes of heredity and evolution. It is not suggested that there is any progressive or other kind of evolution by selection of continuous variations, but it is suggested that the proper appreciation of discontinuous variation, especially in its incipient stages, is possible only if the student knows something of what is involved in critical observations of variation in general. The exercise given below is to be considered only as one possible example, to show the method rather than the data. Many others will occur to the teacher or to the intelligent student, and it would be of more educational value to study quite a different example *along*

the same lines. The notation used is rather unorthodox, and there are many refinements used in research practice which will be found in the standard books on statistics. The aim of this exercise is to teach statistical manipulation only to the extent of first- or second-year work, to show that even crudely quantitative expressions of variations have an interest and significance for the most elementary student of botany.

477. Prepare two blank tables for lengths and widths, like Tables I and II without the measurements. Label at random ten branches of cherry-laurel, each with at least ten leaves on the main stem. Using a millimetre scale, measure to the nearest half-millimetre the full length and the maximum width of each leaf-blade on branch I, entering the data as millimetre numbers in the column I of each table as soon as they are obtained. Measure and record in the same way the length and width of each leaf, from the apex downwards, on each of the other branches from II to X. These recorded measurements give results as in Table I for lengths and Table II for widths.

One hundred individual leaves is rather a small number for statistical purposes and ten branches chosen at random are likely to show rather large errors; but as a student's exercise, the sample taken is at least instructive.

TABLE I

LENGTH IN MILLIMETRES.										
BRANCHES										
	I	II	III	IV	V	VI	VII	VIII	IX	X
1	99	117	101.5	120.5	90.5	108	105	92	109	131
2	125.5	136	115	133	116	129	129	121	123	146
3	142	147	130	143	125.5	143	132	135	137	150
4	145.5	138	118.5	134	136	143	131.5	142	136	121
5	137	120	124	131	139	144	132	149	140	112
6	130.5	117	111	116	137	123	128.5	141	130	105
7	129	115	107	114	134	128	125	139	132	123
8	114	112	84	116	122.5	108.5	112	131.5	110	126
9	102.5	129	101	132	96	125	108	124	131	124
10	134	135	131	126.5	113	140.5	122	127.5	130.5	133

TABLE II

WIDTHS IN MILLIMETRES.

BRANCHES

	I	II	III	IV	V	VI	VII	VIII	IX	X
1	41.5	52.5	40	51	36	45	46.5	34	44	57
2	57	63	53	60	50	55	61	50	58	64.5
3	58	59.5	58	59	59.5	60	57.5	62	57	70
4	59.5	61.5	58	54	53.5	59	57.5	60	56.5	62
5	62	59.5	57	58	55.5	61.5	58	61	55.5	61
6	57	57.5	55	59	55	59	54.5	61	53	48
7	58	56	49	48	53	59	52	54	50	61.5
8	52	49	31	50	51	44	47	55	47	62
9	48	62	49	60	36.5	62.5	44	52.5	56.5	57
10	62.5	61	64	55	51	63	53.5	51	58	57.5

Position and Length. Inspection of Table I will show that the leaves are progressively longer from the apex downwards, for the first three positions on every branch, for the first four positions on branch I, and for the first five positions on branches V, (VI), VIII. After this initial increase, there follows a decrease which is progressive to position 9 on branches I, V and VIII, to position 8 on branch II, to position 7 on branch IV. This decrease also shows irregularly on the other branches.

Position and Width. Similarly inspection of Table II shows that the maximum leaf-width increases from the apex to position 5 on branch I, and to position 3 on branches III, V, VI, VIII, X. The decrease in leaf-width for lower leaves is irregular, but present in a general way.

Position and Size. The recorded measurements show what is apparent to the eye in that the first leaf is smaller, the next medium, and the third larger. There are from seven to nine leaves on each year's growth, so that a small leaf recurs in position 8 to 10, as the top leaf of the previous year. Apart from these obvious correlations of size and position the sizes vary amongst themselves from branch to branch, with the result that we can analyse the distribution of length and width as though we were dealing with a random sample of 100 individuals or "variates." If the first two

leaves on each branch were omitted, the "population" or sample of leaves would be more uniform, and the error of using individuals of different ages would be largely eliminated.

TABLE III

Class Range in mm.	Class Value = V mm.	LENGTH Individuals or Variates	Class Totals = f
I 75-85	80	84	1
II 85-95	90	90·5, 92	2
III 95-105	100	96, 99, 101, 101·5, 102·5	5
IV 105-115	110	107, 108, 108·5, 105, 108, 109, 105, 114, 112, 111, 114, 113, 112, 110, 112	15
V 115-125	120	115, 117, 117, 115, 118·5, 116, 116, 116, 120, 124, 120·5, 122·5, 123, 122, 121, 124, 123, 121, 123, 124	20
VI 125-135	130	125·5, 129, 129, 126·5, 125·5, 129, 128, 125, 129, 128·5, 125, 127·5, 126, 130·5, 134, 130, 131, 133, 134, 131, 132, 134, 132, 131·5, 132, 135, 131·5, 130, 132, 131, 130·5, 131, 133	33
VII 135-145	140	139, 137, 136, 137, 136, 138, 135, 136, 139, 137, 142, 143, 143, 143, 144, 140·5, 142, 141, 140	19
VIII 145-155	150	145·5, 147, 149, 146, 150	5
		Total	100

TABLE IV

Class Range in mm.	Class Value = V mm.	WIDTH Individuals or Variates	Class Totals = f
I 30-35	32·5	31, 34	2
II 35-40	37·5	36, 36·5	2
III 40-45	42·5	40, 44, 44, 44, 41·5	5
IV 45-50	47·5	48, 49, 49, 49, 48, 45, 46·5, 47, 47, 48	10
V 50-55	52·5	52, 52·5, 53, 51, 54, 50, 50, 53·5, 53, 51, 51, 54·5, 52, 53·5, 50, 54, 52·5, 51, 53, 50	20
VI 55-60	57·5	57, 58, 59·5, 57, 58, 59·5, 58·5, 57·5, 56, 58, 58, 57, 55, 59, 58, 59, 55, 59·5, 55·5, 55, 55, 59, 59, 59, 57·5, 57·5, 58, 55, 58, 57, 56·5, 55·6, 56·5, 58, 57, 57, 57·5	37
VII 60-65	62·5	62, 62·5, 63, 61·5, 62, 61, 64, 60, 60, 60, 61·5, 62·5, 63, 61, 62, 60, 61, 61, 64·5, 62, 61, 61·5, 62	23
VIII 65-70	67·5	70	1
		Total	100

478. Variation Polygons. One type of "normal curve of variation" may be considered as the graphic expression of the binomial $(a + b)^n$, where $a = b = 1$ and n is a large number. When n is small, the "curve" becomes an angular line enclosing with the base a "variation polygon." With $n = 6$, the number of individuals or variates is 64, and the

distribution $1 + 6 + 15 + 20 + 15 + 6 + 1$ (see A in Fig. 34). With $n = 7$, the number of variates is 128 and the polygon has a flat top (see B in Fig. 34). In the present example the number of variates is 100, and therefore the polygon should lie between the limits for 64 and 128, with a possibility of an apical angle as in A (64) or C (256) (see Fig. 34).

Grouping. Taking ranges of 10 mm. for lengths and of 5 mm. for widths, group the data in "classes" as in Tables III and IV. The number of individual measurements or "variates" in each class is known as the "frequency." Each class is given a definite "value" in millimetres by taking the mid-point for each class, e.g. the range 95—105 in this case includes all the measurements which are 95 or more up to 104.5, and thus all lie around 100 mm. for Class III lengths. The vertical lines upon which the frequencies are marked pass through the "values" and are thus in the middle of the "ranges."

Graphing. These data from Tables III and IV should be graphed, using the class "values" as abscissæ and the "frequencies" as ordinates. When this is done as in Fig. 34, the polygon outlines are found to fall approximately along the same lines and for the most part between A (64) and B (128). The "peak," or class value with the highest frequency, is known as the *mode*.

The spreading of the lower values and the shortening of the higher values may be due to the method of sampling combined with the correlation and age variation noted above, but the frequencies at the ends are so low that the divergence from the theoretical polygon might not be truly significant.¹

479. Calculation of the "Mean"

The *average length* of leaf in the sample can be obtained by adding together all the values in Table III and dividing by 100, the number of leaves. The *arithmetical mean* can be obtained by adding the extreme values and dividing by two: $84 + 150 \div 2 = 117$. The length of a cherry-laurel leaf, as sampled, is thus 117 ± 33 mm. Similarly from Table IV we get the *arithmetical mean* width $31 + 70 \div 2 = 50.5$, and the maximum width of a cherry-laurel leaf is 50.5 ± 19.5 mm. Since the *modes* for

¹ The degree of significance can be estimated from the relation of the probability integral to the standard deviation, see Fisher, pp. 44-45, also pp. 52 sqq.

length and width occur around 130 and 57.5 there is clearly a need for a better method of calculating the variation around the mean.

The variates have been grouped in "classes," and the usual way of calculating the *mean* is shown in Tables V and VI. Each class value V and each class frequency f is tabulated; these are multiplied across to give a series of $f.V$ data, giving importance to each V in the degree to which it is represented amongst the variates. The $f.V$ data are added together and, using S as a sign for summation, the mean $M = \frac{S(f.V)}{n}$, where n is the total number of variates, for length $M = 125.1$ mm. and for width $M = 55.1$ mm. These values represent the average length and width respectively.

480. Standard Deviation. Having determined the *mean* we are now in a position to calculate the *average deviation* from this mean,¹ but the *standard deviation* gives a more accurate measure of the variation. The derivation of the formula will be found in standard works of statistics; the general principle involves squaring the class deviations from the mean in order to give more importance to the extremes. The formula is for the standard deviation $D = \frac{\sqrt{S(f.d^2)}}{n}$; where d is the class deviation from M the mean (see Tables V and VI). It should be noted that both for length and for width M comes between classes V and VI.

TABLE V

LENGTHS. 100 CHERRY-LAUREL LEAVES.

Classes	V mm.	f	f.V	d	d ²	f.d ²
I	80	1	80	- 45.1	2034	2034
II	90	2	180	- 35.1	1232	2464
III	100	5	500	- 25.1	630	3150
IV	110	15	1650	- 15.1	228	3420
V	120	20	2400	- 5.1	26	520
VI	130	33	4290	+ 4.9	24	792
VII	140	19	2660	+ 14.9	222	4218
VIII	150	5	750	+ 24.9	620	3100
Totals		100	12510			19698

¹ The average deviation = $\frac{S(f.d)}{n}$.

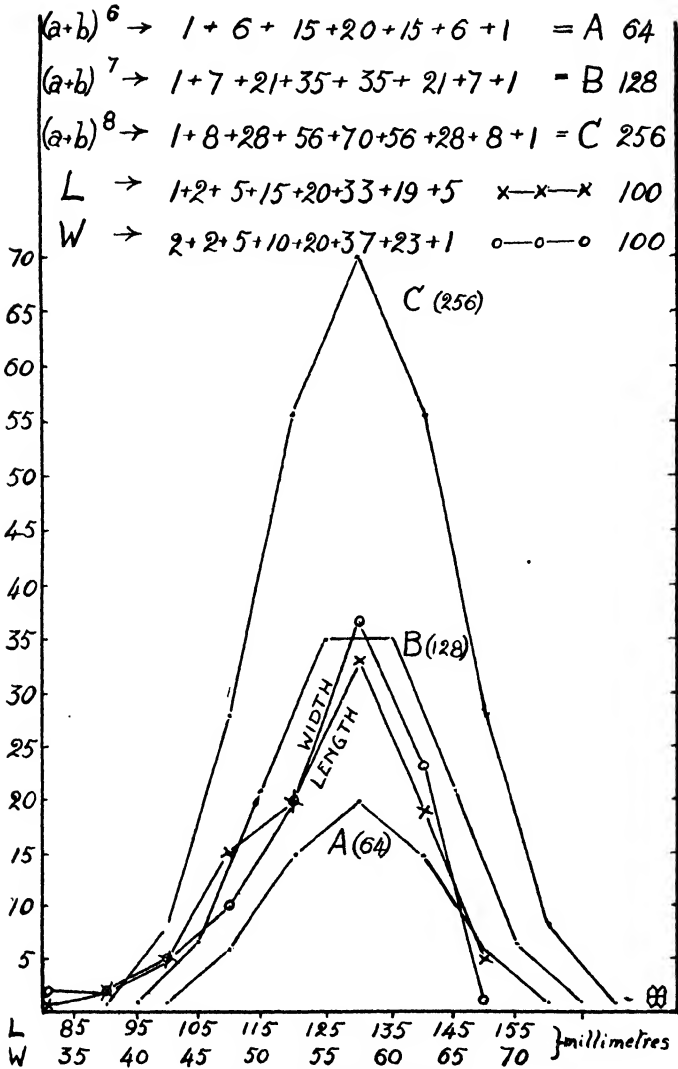


FIG. 84.—Variation Polygons.

EVOLUTION

$$n = 100 \text{ S(f.V)} = 12510$$

$$M = \frac{\text{S(f.V)}}{n} = 125.1 \text{ Mean}$$

$$D = \sqrt{\frac{(\text{f.d}^2)\text{S}}{n}} = \sqrt{\frac{19698}{100}}$$

$$= \sqrt{196.98} = 14.03^1 \text{ Standard Deviation}$$

$$F = \frac{100D}{M} = \frac{1403}{125.1} = 11.21 \text{ Fluctuation}$$

$$\left. \begin{aligned} E_M &= \frac{\pm 0.6745D}{\sqrt{n}} = \pm 0.95 \\ E_D &= \frac{\pm 0.6745D}{\sqrt{2n}} = \pm 0.67 \\ E_F &= \frac{\pm 0.6745F}{\sqrt{2n}} = \pm 0.53 \end{aligned} \right\} \text{ Probable Errors}$$

TABLE VI

WIDTHS. 100 CHERRY-LAUREL LEAVES.

Classes	V mm.	f	f.V	d	d ²	f.d ²
I	32.5	2	65	- 22.6	510.76	1021.52
II	37.5	2	75	- 17.6	309.76	619.52
III	42.5	5	212.5	- 12.6	158.76	792.8
IV	47.5	10	475	- 7.6	57.76	577.6
V	52.5	20	1050	- 2.6	6.76	135.2
VI	57.5	37	2127.5	+ 2.4	5.76	213.12
VII	62.5	23	1437.5	+ 7.4	54.76	1259.48
VIII	67.5	1	67.5	+ 12.4	153.76	153.76
Totals		100	5510			5773

$$n = 100 \text{ S(f.V)} = 5510$$

$$M = \frac{\text{S(f.V)}}{n} = 55.1 \text{ Mean}$$

$$D = \sqrt{\frac{(\text{f.d}^2)\text{S}}{n}} = \sqrt{\frac{5773}{100}}$$

$$= \sqrt{57.73} = 7.6^1 \text{ Standard Deviation}$$

¹ N.B.—These values for *D* show that the grouping used is rather rough since the range or "group interval" should not exceed one quarter of the standard deviation, but with only 100 variates finer grouping is not available.

$$r = \frac{100D}{M} = \frac{760}{55.1} = 13.79 \text{ Fluctuation}$$

$$\left. \begin{aligned} E_M &= \frac{\pm 0.6745D}{\sqrt{n}} = \pm 0.51 \\ E_D &= \frac{\pm 0.6745D}{\sqrt{2n}} = \pm 0.36 \\ E_F &= \frac{\pm 0.6745F}{\sqrt{2n}} = \pm 0.66 \end{aligned} \right\} \text{ Probable Errors}$$

The *standard deviation*, *D*, for length = 14.03 mm. for width = 7.6 mm. These figures obviously do not represent the full deviation from the mean; they may be regarded as average deviations determined rather more accurately than by the simpler method. Geometrically they correspond to the steepest parts of the variation polygons. They give a definite measure in millimetres of the variability in length and maximum width within the selected sample of cherry-laurel leaves and would be found applicable to any other sample *off the same plant*. Other plants differing in variety or in habitat might show different values for *D*, which could be compared like other characters of the plants.

481. Coefficient of Variability. Although we can compare the values of *D* for length in various plants of the same kind, we cannot compare the absolute values (in millimetres) of *D* for length and for width, even in the same plant, since the actual values of *D* are affected by the actual length and actual width which are in ratio of 2 : 1 approximately. A measure of the variability in relation to the value can be obtained by calculating what percentage *D* is of *M*. As this is a measure of the variability or fluctuation we may use *F* as a symbol, instead of the usual notation which does not have any special association-value for a beginner; then the coefficient of variability $F = \frac{100 D}{M}$, for length *F* = 11.21 and for width *F* = 13.79. These values are now comparable and we might say that the length varies a little less than the maximum width in cherry-laurel leaves, but see below, Ex. 482.

482. Probable Errors. The theory of error will be found in standard works on statistics; the only points of general principle which we need to note here are, first, that the reliability of the results increases not directly as the number of individuals measured, but directly as the square root of that number. Thus if we desired to double the reliability of our present data which concern 100 variates, we would find it necessary to measure twice ten squared or 400 leaves. Measuring twice the number of

leaves would increase the reliability only by about 40 per cent., so that 100 is a practically convenient number.

The formula for calculating probable errors of the mean E_M , the standard deviation E_D and the coefficient of variability E_F are given with the cherry-laurel values in Tables V and VI. The formula for E_F requires a correction when F is higher than 10, but the simple formula is sufficiently accurate for present purposes.

The probable errors in Table V and VI are all low compared with their corresponding constants, so that we can consider these more or less correct.

The conclusion drawn at the end of Ex. 481 requires further examination in view of the fact that the actual difference of 2.58 may not be significant.

The Probable Errors of Differences. This is found by squaring the E_F values for length and for width, adding the results and taking the square root of the sum; thus $(0.53)^2 + (0.66)^2 = 0.7165$ and the square root of 0.7165 = 0.85. This should be compared with 2.58 as the actual difference found, see also below.

Probable Errors and Significance of Results

Mathematically the chances of the result being correct are even when the result and the probable error have equal values. The chances of the result being correct increase rapidly when the datum value rises to 2E (4.6 to 1), 3E (21 to 1), 4E (142 to 1), 5E (1,810 to 1), 6E (19,200 to 1). Although an even chance (1 to 1) may be a *sporting* "certainty" on the totalisator, the statistician would regard the result, win or lose, as having no significance. Even when the chances rise to 3E or 21 to 1, he is still doubtful. Datum values above 3E are generally regarded as significant, but the degree of significance varies with the datum considered.

It is quite clear, therefore, that the present set of measurements giving a difference of 2.58 in the coefficients of variability for length and maximum width in cherry-laurel leaves, cannot be said to prove *definitely* that length is less variable than maximum width, since the probable error of the difference is 0.85. The application of such a check upon the significance of results is very useful in all biological investigations. The repetition of the same result for an experiment is clearly required up to three or four times before the result can be said to be significant; even with more repetition it is still possible to draw wrong conclusions from the results.

483. Correlation. This analysis of length and maximum width of cherry-laurel leaves has, so far, yielded results which may be regarded as characters of the sample or of the shrub, and these become interesting only when compared with

similar characters in other samples or shrubs. We have, however, two sets of data and although we have been unable to prove length more variable than width, we may proceed further and calculate the degree to which maximum width is correlated with length.

It is obvious, of course, that the larger the leaf the greater

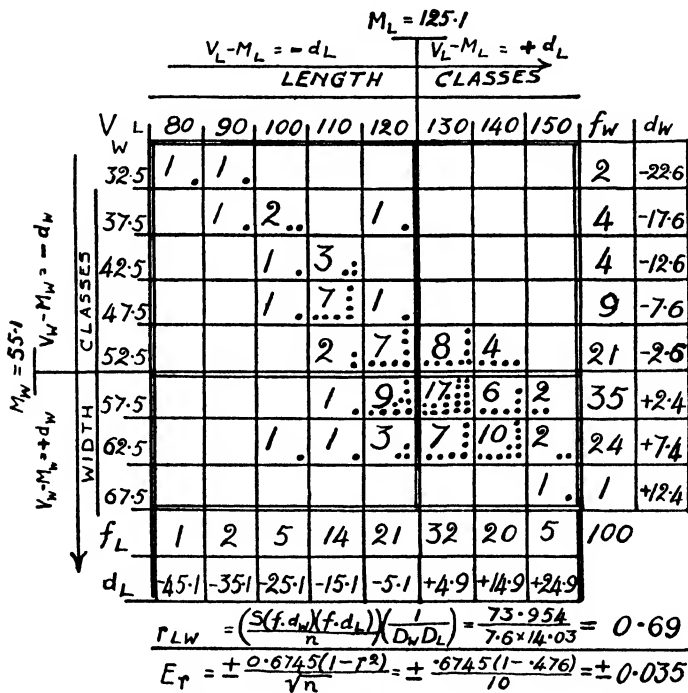


FIG. 35.—Correlation Dot Diagram and Table (see p. 208).

its length and width, but it is comparatively easy to find leaves of equal width which differ in length or equal length which differ in width.

A correlation dot diagram (see Fig. 35) is prepared by making vertical columns for one set of classes, e.g. lengths, and a crossing set of rows for the other set of "classes," e.g. widths. Then by reference to Tables I and II each leaf is tallied, by means of a dot, into its appropriate square.

The class values for length are 80, 90, etc., which means that a leaf with a length between 75 and 84.5 (in practice) is tallied in the first vertical column, while if the same leaf has a width between 30 and 34.5 (in practice) it is tallied in the first horizontal row; and the dot is put in the left top corner. When all the hundred leaves have been tallied as dots, the latter can be counted and these numbers (frequencies) used for calculation. The dot diagram becomes a correlation table when numbers are used. The distribution of the dots mainly in left top and right bottom quadrants indicates at once a high degree of positive correlation. The frequencies are tabulated below for length and to the right for width. The calculated means are indicated by lines at right angles (see Fig. 35), M_L and M_W . The deviations from the M_L and M_W of the class values V_L and V_W are similarly tabulated.

A formula for the *coefficient of correlation* r_{LW} is given in Fig. 35. In practice the summation of the (f.d_W) (f.d_L) values is a little complicated, therefore it is given in detail below¹:

Classes	(d _W) (f.d _L)	
I	- 22.6 [- 45.1 + (- 35.1)]	= + 1812.5
II	- 17.6 [- 35.1 + (- 50.2) + (- 5.1)]	= + 1591.0
III	- 12.6 [- 25.1 + (- 45.3)]	= + 887.0
IV	- 7.6 [- 25.1 + (- 105.7) + (- 5.1)]	= + 1032.8
V	- 2.6 [- 30.2 + (- 35.7) + (+ 39.2) + (+ 59.6)]	= - 85.5
VI	+ 2.4 [- 15.1 + (- 45.9) + (+ 83.3) + (+ 89.4) + (+ 49.8)]	= + 387.6
VII	+ 7.4 [- 25.1 + (- 15.1) + (- 15.3) + (+ 34.3) + (+ 140) + (+ 49.8)]	= + 1314.2
VIII	+ 12.4 [+ 24.9]	= + 308.8
S (f.d _W) (f.d _L)		= + 7248.4

S (f.d_L) (f.d_W) is then divided by the product of the two standard deviations, *i.e.* by $D_L \times D_W$. The result in this case is 0.68, but before we can say whether this is significant or not, the probable error Er must be known. The formula is given in Fig. 35 and is:

$$Er = \pm \frac{0.6745 (1 - r^2)}{\sqrt{n}} = \pm 0.036.$$

When the value of a correlation coefficient is "more than six times the size of the probable error the existence of correlation is a practical certainty. When Er is small, and r is less than 0.3, correlation is not marked; when r is above 0.5 there is decided correlation."²

¹ The values for the total, the coefficient and the probable error given in Fig. 35 are all slightly wrong.

² See King in "Genetics in Relation to Agriculture," by Babcock and Clausen. McGraw-Hill Book Company.

In the present case r_{LW} is 0.68 and $Er \pm 0.036$, so that on both counts there is decided evidence of correlation between length and maximum width in cherry-laurel leaves.

The student will find that these exercises, 477 to 483, take less time to do than to explain. If he will only take the trouble to start an analysis of this kind on different material, he should find the exercises quite fascinating. Further, he will at the end be practically acquainted with the rudiments of statistical analysis and able later on to appreciate these tests of accuracy as applied by others in an increasing degree to the results of biological investigations.

484. Mixed Populations and Mutations. When much larger numbers of variates are considered, so that the variation *polygon* should approach the normal *curve*, and the normal grouping shows two *modes*, or peaks, of which one may be much smaller than the other, then a mixed population is indicated. The values of M , D and F for such a mixed sample would be liable to large errors and of little significance, but by careful selection the two groups might be separated and their statistical constants compared.

(a) This might be done, for example, with a mixture of two strains of maize or broad bean, the lengths and/or weights being taken for the mixed population which could afterwards be separated by another character such as colour.

(b) Mixed samples of caraway seeds, measured for length and maximum width by means of a pocket-lens scaleometer make very suitable material.

Although quite beyond the practical scope of this book, it should be noted that by such studies applied to proved pure lines or uniform populations the first beginnings may be detected of changes which, within one or two seasons, may grow to be quite large differences, converting the pure line into a mixed population in a very short time and giving rise by mutation to new forms or varieties.

The following are useful books of reference :—

“Genetics in Relation to Agriculture,” especially Chapter III, by E. B. Babcock and R. E. Clausen. McGraw-Hill Book Co.

“Statistical Methods for Research Workers,” by R. A. Fisher. Oliver and Boyd.

“An Introduction to the Study of Statistics,” by G. Udny Yule. Griffen. London.

CHAPTER XXIX

ECOLOGY

ELEMENTARY practical exercises in ecology may be conveniently divided into three sections—namely, ecological anatomy, laboratory examination of soils, and field work.

ECOLOGICAL ANATOMY

485. Sun and Shade Leaves. Collect two sets of leaves from the same tree, *e.g.* poplar, beech, etc., one set from the part most exposed to sunlight and the second set from the part in the deepest shade available. Examine representative leaves from each set and tabulate in two columns all the differences which you can detect between the two sets (a) without a microscope, and (b) in sections examined with the aid of a microscope. Make annotated drawings of characteristic anatomical features (see Figs. T 883–884).

XEROMORPHIC CHARACTERS

Since critical investigations do not always confirm the apparent efficiency of characters which appear to be xerophytic, it is advisable to consider the anatomy of apparently xerophytic plants under the above heading.

486. Marram Grass. (a) Examine a leaf of *Ammophila arenaria*; note the ridges on the upper (adaxial) surface; examine a cross-cut surface under a pocket-lens and note the stiff hairs upon the ridges which are white internally, with dark green arcs in the furrows between the ridges.

(b) Examine a thin section of the above leaf; make a line drawing to show the distribution of abundant sclerenchyma, slightly developed chlorenchyma, hairs and motor cells, together with the vascular bundles (see Fig. T 893). Draw two ridges and one furrow under the high power of the microscope. See also Ex. 492.

487. Examine, recording your observations in the same

way as above (see Ex. 486), one or more of the following examples of **dry xeromorphy**—stem of broom, gorse ("P.-l. P. L.," 177-178); stem and phylloclade of *Ruscus*; leaf of rosemary, oleander, pine, *Hakea*, *Calluna*, *Erica*, *Empetrum*, *Nardus stricta*, *Festuca rubra* (see Figs. T 892, 890, 889, 891 and "P.-l. P. L.," No. 85, 86, 158).

488. Succulent Xerophytes. Examine macroscopically, and with the aid firstly of a pocket-lens and secondly of a microscope used on appropriate sections, one or more of the following examples of succulent xerophytes—leaf of *Crithmum maritimum*, *Aloë* (Fig. T 1334), *Puya*, *Bryophyllum*, *Semprevivum*, *Crassula*, *Echeveria*, *Mesembryanthemum*, *Haworthia*; shoot of *Phyllocactus*, *Cereus*, *Mammillaria*, and other cacti, also *Kleinia articulata*, *Stapelia*. Note the presence or absence of reduction in leaf surface, the sunk stomata, the water-storage tissue and any other characteristic features; record your observations by means of line diagrams and drawings under H.P. of the smaller details.

OTHER TYPES

489. Halophytes. Since these are plants which occur in physiologically dry habitats, they show many of the xeromorphic characters studied in the above exercises. Examine and make annotated drawings of the characteristic xeromorphic features of the leaf of *Armeria maritima*, *Atriplex portulacoides* (dry); the shoot of fresh *Salicornia europæa*, the leaf of *Salsola*, *Suaeda*, *Plantago maritima*, *Triglochin maritimum*, *Cochlearia officinalis* (succulent). The morphology and structure of *Salicornia* is rather special; briefly, the rather thin woody stem is completely covered by succulent leaf-bases which pass mainly downwards from the nodes; for details see E. de Fraine in *Jour. Linn. Soc.*, 41, 317, 1913.

490. Hydrophytes. The soft tissues of many of these plants make it rather difficult to prepare satisfactory permanent preparations. The glycerine jelly method meets most of the difficulties. A very sharp razor is used and reasonably thin sections are taken; the best section is chosen and floated out in abundant water on a slide. If necessary a brush may be used to arrange the section in the middle of the slide, then the excess water is carefully removed. Two drops of melted glycerine jelly, made up with eosin or methylene blue so that it contains about 0.1 per cent. of either dye, is

dropped on the section and a cover-slip is added before the staining jelly-mountant cools. The preparation is then set aside for two or three weeks, at the end of which the section will be found to have taken up the dye from the mountant jelly, then the tissues can be distinguished clearly. If differential staining is desired, aqueous methylene blue can be used on the section before it is mounted in eosin jelly. Using the above method, prepare transverse sections of the stems of representative hydrophytes. Make line diagrams of these sections and H.P. drawings of characteristic details. Suitable plants are *Potamogeton*, *Nymphaea*, *Hippuris* (see Figs. 895-896). Note particularly the large development of aerenchyma, also the slight development of conducting tissues and lignification.

Comparisons

491. Write a comparative account, on the basis of your own observations, of a typical dry xerophyte, a typical succulent xerophyte, a halophyte and a hydrophyte.

492. Extend Ex. 486 to include an investigation of the rhizome and the subterranean parts of the leaves of marram grass. Note the aerenchyma of the leaf-sheath and compare the subterranean parts of the plant with the xeromorphic upper parts.

493. Cut and mount in glycerine jelly a vertical section of a floating leaf of water-lily. Compare carefully the xeromorphic upper zone with the hydrophytic lower zone of the section. Make an annotated line diagram of the characteristic features and write an account of this type of leaf in relation to its two distinct habitats.

494. **Aerenchyma.** The development of an efficient aerating system is characteristic of plants which grow under water (hydrophytes), also of many marsh plants and hygrophytes. The lower and upper parts of such plants may be rather different. Investigate the anatomy of the lower and upper parts of one or more of the following: *Ulmaria palustris*, *Campanula rotundifolia*, *Juncus communis*, *Caltha palustris*, *Phragmites*.

495. **Hydathodes.** Examine with a pocket-lens, and also by means of sections under the microscope, the hydathodes or water-secreting glands in one or more of the following: old and very young leaves of *Saxifraga umbrosa*, or other species of saxifrage, *Alchemilla vulgaris*, *Aconitum napellus*, *Potentilla*, *Geum*, *Delphinium*, *Tropæolum majus*, *Helleborus*

niger, *Ulmus campestris*, *Vicia sepium*, *Primula*, *Papaver*, *Geranium*, *Fuchsia*. These are to be found mainly at marginal vein-ends, but *Phaseolus vulgaris*, *Piper nigrum*, etc., have trichome-hydathodes scattered on the leaf surface. Stomatal hydathodes may be found on the leaves of *Aconitum*, *Tropaolum*, *Hippuris*, *Callitriche*, *Empetrum nigrum*, *Papaver* and *Colocasia*. Apical hydathodes occur at the tips of many young grass leaves, e.g. wheat, rye, maize. See also Ex. 78.

SOILS

496. Make a comparative examination under the microscope of the following **types of soils**, mounted in water, noting the main characteristics and measuring the size of the large particles in the siliceous types :—

Coarse sand—1·0 to 0·2 mm. diameter; translucent or opaque, mainly sharply angular fragments when water borne; with rounded corners when wind borne.

Fine sand—0·2 to 0·04 mm. diameter; as above with no rounded corners.

Silt—0·04 to 0·01 mm. diameter; as above with flat particles.

Fine silt—0·01 to 0·005 mm. diameter; as above with more numerous minute flakes.

Clay—less than 0·005 mm. diameter; in flat particles or flakes, and granules; shows Brownian movement.

Chalk—amorphous or showing shell fragments, opaque white or variously coloured.

Loam—a mixture of the above types, with humus.

Peat—black or brown fragments of plants, sometimes with recognisable pieces of *Sphagnum* leaves, and often with recognisable pollen grains (see Fig. 25).

497. Sedimentation. Prepare three separate samples of different soils, e.g. sand, clay and garden loam, and three corked test-tubes at least 15 cm. long. Place equal quantities (about 2 grammes) of soil in the three tubes; add distilled water; cork and shake thoroughly. Set the three preparations aside in a quiet place and note the rate at which sedimentation takes place. Mark a point on each tube 2 cm. from the water surface and record the time in which the water becomes clear down to the mark.

498. Flocculation. Rub up 4 grammes of clay with 10 c.c. distilled water; transfer the suspension in equal

parts to two long test-tubes ; fill up one tube with distilled water and the other tube with lime water ; cork ; set aside ; and note the rate of sedimentation as before. The lime water "floculates" the clay ; compare this action with that of salts on ferric hydroxide sol, Ex. 384. In both cases a surface electric charge is neutralised, leading to aggregation of the colloidal particles, but the actual mechanism of neutralisation appears to be different ; clay acts as a weak acid ; the particles are negatively charged and are "neutralised" by a base such as calcium hydroxide.

499. Capillarity. Prepare four glass tubes of at least 2 cm. bore and 60 cm. length. Tie muslin round one end of each tube ; pack carefully and uniformly into the four tubes dry powdered samples of sand, clay, loam and peat. Fix all four tubes vertically with the muslin ends under 2 cm. water. Note and compare (a) the rates at which the various soils become wet, and (b) the heights of the wet parts of the columns when equilibrium is attained. Both the rate and the limit of capillary flow are important in natural soils, where the conditions are, of course, seldom or never the same as in the above experimental arrangement, which is purely comparative and artificial.

500. Percolation. The draining power of soils depends upon their permeability or the rate at which water can percolate through the medium. Using four tubes, as in Ex. 499, but carefully packed with *water-saturated* samples of the four types of soil, determine the time taken in each case for the downward passage of 100 c.c. water, which should be poured into a clear upper 5 cm. zone of the tube gradually, in such a way that there is free water above the soil until all the 100 c.c. quantity has been used. The success of this comparison depends very largely on the care taken in packing the tubes uniformly, but this process and the necessary precautions will be familiar to all pharmaceutical students. The soil samples should be added gradually and tamped down carefully after each small addition.

501. Evaporation. Using the same four types of soil, again in the saturated condition, pack four similar Petri half-capsules at least 2 cm. deep and about 10 cm. wide with soil so that a flat surface as smooth as possible is exposed at the top. Weigh the various preparations ; set them aside, all under the same conditions of temperature, relative humidity and air movement. Without disturbing

the surfaces in any way, weigh the preparation again after four hours, after eight hours, and at daily intervals for a week. Compare the four samples as regards (a) the rate of evaporation, and (b) the total evaporation in a week. Compare and record the condition of the dried surfaces.

For further exercises on soils see "Practical Plant Ecology," by Professor A. G. Tansley.

FIELD WORK

502. Sketch a characteristic **wind-form** of hawthorn, sea buckthorn or other large shrub or tree. Examine the specimen carefully and note the effects of wind action on the windward buds as compared with the leeward branches.

503. Examine and sketch wind-forms of other plants, such as wind-cut heather, heath and juniper.

504. Note and compare the occurrence of trees along the banks of streams in moorland or grassland and the occurrence of normal mesophytes about fresh-water streams passing through a pebble beach. Both are examples of a "higher" association within a "lower" association, both produced as the effect of additional water supply.

SURVEY

For those who wish to enjoy detailed ecological field work there is no better guide than "Practical Plant Ecology," by Professor A. G. Tansley; there the student will find all the necessary directions for making preliminary surveys, garden and field quadrats, line and belt transects, profile and bisect charts, etc. "Types of British Vegetation," by the same author, is the standard book on British Formations.

Those who desire only to recognise in the field the general groups of plants or kinds of vegetation *in these islands* may find the following incomplete floristic lists useful. These lists are arranged in series of progressive associations, e.g. **hydroseres**¹ from aquatic to fen and lowland moor formations, and from salt water through brackish water to mud-flat and

¹ A hydrosere is a progressive series of associations where the edaphic wetness is gradually reduced by the continued *action of the vegetation*, e.g. by raising the level of the soil. Similarly a xerosere is a progressive series of associations where the edaphic *dryness* is gradually reduced by the *action of the vegetation*, e.g. by binding loose sand or forming soils over bare rock surfaces.

salt-marsh ; **xeroseres**¹ from the dry halophytic foreshore to sand-dune backs, and from lichens on rocks to sub-alpine grasslands. The main elements of siliceous and calcareous associations are also noted.

Inland Hydrosere

AQUATIC FORMATION

- I. **Submerged Leaf Association.** *Elodea canadensis*, *Potamogeton* spp., *Utricularia* spp., *Myriophyllum spicatum*, *Ceratophyllum*, *Hippuris*, *Stratiotes*, *Apium* spp., *Cenanthe fistulosa*, *Zannichellia*, *Hottonia*, *Elatine* spp.
- II. **Free Floating Leaf Association.** *Lemna* spp., *Hydrocharis morsus ranæ*.
- III. **Rooted Floating Leaf and Open Reed Swamp Association.** *Typha*, *Phragmites*, *Scirpus*, *Nymphaea*, *Castalia*, *Potamogeton*, *Ranunculus* spp., *Sagittaria*, *Glyceria fluitans*, also *Carex* spp.
- IV. **Closed Reed Swamp Association.** *Typha*, *Phragmites*, *Phalaris*, *Glyceria aquatica*, or *Scirpus lacustris* as dominants, with *Utricularia*, *Myriophyllum*, *Ranunculus lingua*, *Radicula amphibia*, *Epilobium hirsutum*, *Cicuta virosa*, *Apium inundatum*, *Sium latifolium*, *Cenanthe* spp., *Lysimachia vulgaris*, *Myosotis palustris*, *Menyanthes*, *Stachys palustris*, *Mentha* spp., *Rumex hydrolapathum*, *Polygonum amphibium*, *Iris pseudacorus*, *Sparganium* spp., *Acorus*, *Alisma plantago*, *Butomus*, *Carex riparia*, *C. stricta*, *Equisetum limosum*.

FEN FORMATION

- I. **Fen Association.** Dominants: *Phragmites*, *Cladium*, *Calamagrostis canescens*, *Juncus obtusiflorus* ; forming societies: *Phalaris*, *Glyceria aquatica*, *Carex* spp., *Eriophorum angustifolium*, *Poa trivialis*, *Iris pseudacorus* ; some other plants : *Anagallis tenella*, *Drosera intermedia*, *Galium palustre*, *Hydrocotyle*, *Lathyrus palustris*, *Myrica gale*, *Cenanthe fistulosa*, *Ophioglossum*, *Potentilla palustris*, *Rhamnus frangula*, *Thalictrum flavum*, *Ulmaria palustris*, *Valeriana officinalis*.

¹ See footnote on previous page.

Intermediate to Moor. *Molinia cærulea*.

- II. **Swamp Carr Associations.** *Alnus, Salix cinerea*.
 III. **Fen Carr Association.** *Alnus, Salix cinerea, Rhamnus catharticus, R. frangula, Viburnum opulus*, with *Myrica, Salix repens, Ligustrum, Ribes* spp., *Fraxinus, Betula tomentosa* or *B. pubescens, Quercus robur* and herbs of the fen association.
 IV. **Ultimate Carr Associations** from II. or III.: as for Fen Carr with *Alnus* dominant, also *Urtica dioica, Iris, Carex* spp., *Lastræa thelypteris, Osmunda regalis*.

LOWLAND MOOR FORMATION

This develops through the *Molinia* stage to *Sphagnum, Eriophorum, Calluna*, etc.; the stages and floristic composition being similar to those in the Upland Moor Formation, which see.

- I. **Molinietum.** *Molinia cærulea* dominant, with *Eriophorum angustifolium, Erica tetralix, Carex inflata, C. flava, C. panicea, C. goodenowii, Potentilla tormentilla, P. palustris, Viola palustris* and occasionally with *Sphagnum* spp. and *Drosera rotundifolia*, also *Triodia decumbens, Lycopus, Scutellaria* and other herbs of the fen.
 II. (a) Mixed *Carex* with *Calluna*.
 (b) **Eriophoretum** }
 III. **Callunetum** } see Upland Moor Associations.

Estuarine Hydrosere

SALT-MARSH FORMATION

- I. **Grass-wrack Association.** *Zostera* open; pure or with occasional *Salicornia*.
 II. **Glasswort Association.** *Salicornia europæa* open; pure or with some *Zostera*. With mud the first associates are *Glyceria* and *Triglochin* or *Aster*; with sand *Salicornia radicans, Atriplex portulacoides* with *Glyceria* and others.¹
 III. **Glycerietum maritimæ.** *Glyceria maritima*, at first in

¹ *Spartina* spp. replace glasswort at various places as mud colonisers, notably between Southampton and Studland.

patches, then as a closed turf; pure or with glasswort, sea lavender, sea pink, sea blite, or scurvy grass.

IV. **General Salt-marsh Association** includes *Armeria maritima*, *Aster tripolium*, *Plantago maritima*, *Triglochin maritimum*, *Glyceria maritima*, *Spergularia salina*, *S. marginata*, *Atriplex portulacoides*, *Limonium vulgare*, *Cochlearia officinalis*, with sometimes *Artemisia maritima*, *Salicornia radicans*, *S. europæa*, *Suaeda maritima*, *Juncus gerardi*.

(a) **Armerietum** with *Glyceria*, lower;

(b) **Armerietum** with *Festuca*, higher; this passes to ordinary pasture above.

V. **Sea-rush Association.** *Juncus maritimus* with *Festuca rubra* var. *arenaria*, *Agrostis palustris* and other grasses, also *Glaux maritima*, *Plantago coronopus*, *P. maritima*, *Cenanthe lachenalii*, *Agropyron pungens*.

Maritime Xerosere

SAND-DUNE FORMATION

I. **Strand Associations.** (a) *Arenaria peploides*, *Salsola kali*, *Polygonum raii*, *Atriplex patula*, *A. hastata*, *A. babingtonia*, *Crambe maritima*, *Cakile maritima*.

(b) *Agropyron junceum*, pure, open.

(c) Incipient dunes—with dune builders as below.

II. Dune-building Associations

(a) **Ammophiletum.** *Ammophila* (*Psamma*) *arenaria*, pure on the seaward side or with *Pteris aquilina*, *Ononis repens*, etc.

(b) **Dunes.** *Ammophila* dominant; on the top and landward faces including *Phleum arenaria*, *Aira præcox*, *A. caryophyllea*, *Festuca rubra* var. *arenaria*, *Galium verum*, *Leontodon nudicaule*, *L. autumnale*, *Hypochaeris glabra*, *Hieracium umbellatum*, *H. pilosella*, *Taraxacum erythrospermum*, with sometimes *Eryngium maritimum*, *Carlina vulgaris*, also *Hippophaë rhamnoides*, *Euphorbia paralias*, *Peltigera canina*, *Erophila verna*, *Cerastium semidecandrum*, *Erodium cicutarium*, *Viola tricolor*, *Lotus corniculatus*, *Trifolium* spp., *Centaurium vulgare*, *Thymus serpyllum*, *Alchemilla arvensis*, *Thalictrum minus*, *Carex arenaria*, *Sedum acre*, *Calystegia sol-*

danella, *Jasione montana*, *Geranium molle*, *Linum catharticum*.

(c) **Lyme-grass Association.** *Elymus arenarius* dominant.

(d) **Sand-sedge Association** similar to above, may initiate (a) or (b) on the seaward side.

III. Dune-back Associations

(a) **Creeping Willow Associations**; dry or wet: *Salix repens* dominant; with many mosses: *Carex* spp., *Blackstonia (Chlora) perfoliata*, *Lotus corniculatus*, *Pyrola rotundifolia* in dry places; and *Parnassia palustris* in wet places.

(b) **Dune Marsh Associations.** *Carex* spp., *Hypnum* spp., *Juncus maritimus*, *Parnassia*, *Anagallis tenella*, *Hydrocotyle*.

(c) **Dune Heath Associations.** *Calluna vulgaris* abundant; with *Erica cinerea*, *Rosa spinosissima*, *R. eglanteria*, *Rubus discolor* (agg.), *Viola tricolor*, *V. ericetorum*, *Teucrium scorodonia*, *Polygala vulgaris* (agg.), *Potentilla reptans*, *P. anserina*, *Sambucus nigra*, *Crepis virens*, *Cnicus arvensis*, *Myosotis collina*, *Veronica chamaedrys*, *Plantago lanceolata*, *P. coronopus*, *Achillea millefolium*, *Anagallis arvensis*, *Sonchus arvensis*, *Spergularia rubra*, *Lithospermum officinale*, *Lycopsis arvensis*, *Valerianella olitoria*, *Hypericum perforatum*, *Poa* spp.; other woodland plants may occur such as hogweed, bluebell, wood avens, wild strawberry, wood sorrel, anemone.

UPLAND MOOR FORMATION

The same species in various degrees of frequency or abundance recur, and contractions are used below. In order of decreasing water-content there are:—

I. **Bog-moss Association.** *Sphagnum recurvum* and other species dominant; with *Erica tetralix*, *Oxycoccus quadripetala*, *Eriophorum angustifolium*, *Empetrum nigrum*, *Calluna vulgaris* and *Rubus chamaemorus*.

II. **Cotton-grass Associations.** *Eriophoretum vaginati* and *Eriophoretum angustifolii*: *E. vag.* or *E. ang.* separately dominant or co-dominant; with *Calluna*, *Empetrum*, *Erica t.*, and *Vaccinium myrtillus* locally abundant; also *Rubus chamaemorus*, *Molinia caerulea*, *Scirpus*, *Carex curta*, *Narthecium ossifragum*, *Oxycoccus*, *Pinguicula vulgaris*, *Andromeda polifolia*.

III. **Grass-moor Associations.** (a) *Molinietum cæruleæ* with *Parnassia* and other hygrophytes.

(b) *Nardetum strictæ* with *Agrostis tenuis* and *Juncus squarrosus* co-dominant; also *Scabiosa succisa*, *Antennaria dioica*, *Euphrasia*, *Prunella*, *Anthoxanthum*, *Agrostis canina*, *Deschampsia flexuosa*, *Holcus lanatus*, *Sieglingia*, *Briza media*, etc.

IV. **Deer-grass Association.** *Scirpus cæspitosus* dominant; with frequent *E. ang.*, *Calluna*, *Erica t.*, *Narthecium*, *Sphagnum*, *Drosera rotundifolia*, *Andromeda*, *Rhacomitrium lanuginosum*, *Cladonia rangiferina*.

V. **Heather Moor Association**

Calluna vulgaris dominant; with frequent *V. myrtillus*, *V. vitis-idaea*, *Empetrum*, *Erica cinerea*, also *Juncus squarrosus*, *Deschampsia flexuosa*, *Galium saxatile*, *Ulex gallii*, *Agrostis* spp., and *Pteris aquilina*: occasional are *Polygala serpyllacea*, *Lathyrus montanus*, *Potentilla sylvestris*, *P. tormentilla*, *Hieracium pilosella*, *Campanula rotundifolia*, *Linum catharticum*, *Rumex acetosella*, *Luzula erecta*, *Scirpus*, *Carex glauca*, *Molinia*, *Festuca ovina*, *Nardus stricta*, *Blechnum spicant*; bryophytes and lichens include *Dicranum scoparium*, *D. majus*, *Campylopus flexuosus*, *Hypnum purum*, *H. cupressiforme*, *Hylocomium squarrosus*, *Grimmia apocarpa*, *Polytrichum* spp., *Ptychomitrium polyphyllum*, *Rhacomitrium* spp., *Sphagnum* spp., *Thuidium tamariscinum*; *Cladonia* spp., *Lecanora* spp., *Peltigera canina*.

Societies of Stagnant Hollows include in addition to some of the above:

Oxycoccus, *Narthecium*; *Juncus* species *acutiflorus*, *lamprocarpus*, *supinus*, *articulatus*, *Carex* species *curta*, *flava*, *panicea*, *stellulata*, *goodenowii*; *Ranunculus flammula*, *Viola palustris*, *Cnicus palustris*, *Taraxacum paludosum*, *Pedicularis sylvatica*, *Eriophorum* vag., *Pinguicula vulgaris*, *Drosera r.*, *Orchis ericetorum*, *Andromeda*; *Montia fontana* occurs with running water.

VI. **Bilberry Moor Association.** *Vaccinium myrtillus* dominant; *Empetrum* and *Calluna* abundant; occasional are *Nardus*, *Deschampsia flexuosa*, *Festuca ovina*, *E. ang.*, *Juncus squarrosus*, *Rumex acetosella*; with rarer *E. vag.*, *Lycopodium* spp., *Rubus chamaemorus*.

This occurs on ridges and other well-drained slopes or summits.

Summit Associations. Tracing the xerosere from the rocky mountain top we have the following :—

MOUNTAIN TOP DETRITUS FORMATION

I. **Lichen Association.** *Lecanora* spp., *Lecidea* spp. on rocks followed by *Andreæa* spp., *Grimmia* spp., etc.

II. **Moss-lichen Association.** *Rhacomitrium lanuginosum* dominant; with *Cladonia* spp., *Deschampsia*, *Festuca ovina*, *Nardus stricta*, *Lycopodium selago* and occasional *Empetrum*.

Many other species occur locally.

III. **Sub-alpine Associations.** (a) *Rhacomitrium* Heath: in wetter places, II. develops towards *Callunetum*¹ floristically, with additional arctic or sub-arctic, such as *Arctostaphylos uva-ursi*, *A. alpina*, *Cerastium arcticum*, etc.

(b) *Rhacomitrium* Moor: in better-drained places, II. develops towards *Vaccinietum* floristically, with *V. vitis-idaea*, *E. vag.*, *Scirpus c.*, *Calluna*, *Empetrum*, etc., with *Trientalis europæa* as a northern species.

CHOMOPHYTE FORMATION

I. and II. above may pass into the associations of ledges and corries; these rather local communities are described in Tansley's "Types of British Vegetation." The various exposed, shaded, hydrophilous, and sheltered stages pass at lower altitudes into the:—

IV. **Arctic-alpine Grassland Association:** with *Festuca ovina*. *f. vivipara* dominant; also *Alchemilla alpina*, *Luzula spicata*, *Poa alpina*, *Phleum alpinum*, *Lycopodium alpinum*, *L. selago*, *Selaginella selaginoides*, etc.

V. **Sub-alpine Grasslands.** These occur below IV. and approach floristically the summit heaths of lower mountains with co-dominants *Festuca ovina*, *Agrostis tenuis*, *Deschampsia flexuosa*, *Anthoxanthum odoratum*; also *Nardus stricta*, *Molinia caerulea*, *Carex goodenowii*; many of the species given under *Callunetum* and *Dune-back Heath*; with *Hypericum pulchrum*, *Potentilla erecta*, *Veronica serpyllifolia*, *Orchis maculata*, *Aira cæspitosa*, and *Botrychium lunaria* as other elements.

¹ For *Callunetum* sub-associations, see *Proc. R.I.A.*, 39, pp. 441-442, 1930.

IV. and V. pass into upland moors at lower altitudes. Other formations are given very briefly because they are well known, but often less easily accessible than the open coast or mountain regions.

FORMATION OF CLAYS AND LOAMS

- I. **Quercetum roboris.** Oakwood association with *Quercus robur* dominant; other trees—hazel, hawthorn, blackthorn, dogwood, and guelder rose; *Rubus* spp., anemone, buttercups, bluebell, wood-sorrel, avens, wild strawberry, wood sanicle, and many other characteristic plants.
- II. **Neutral Grassland Association:** with dominant grasses *Lolium perenne*, *Cynosurus cristatus*, *Anthoxanthum odoratum*, *Dactylis glomerata*, *Holcus lanatus*, *Alopecurus pratensis*, *Bromus mollis*, *Festuca duriuscula*; other grasses—*Agrostis tenuis*, *A. alba*, *Poa pratensis*, *P. trivialis*, *Phleum pratense*, *Arrhenatherum elatius*; and many herbs.
- III. **Juncetum communis.** This association of marshy meadows includes *Juncus conglomeratus* with *J. effusus*, *J. articulatus*, *J. glaucus*, *J. bufonius* and many marsh herbs.

FORMATION OF SANDY SOIL

- I. **Quercetum arenosum.** Dry oakwood association with *Q. robur* and *Q. sessiliflora* separately dominant or co-dominant; with *Holcus mollis*, bluebell, anemone, wood-sorrel, etc.
- II. **Grass-heath Association** with *Anthoxanthum*, *Agrostis canina*, *A. setacea*, *A. tenuis*, *Aira præcox*, *A. caryophyllea*, *Deschampsia flexuosa*, *Sieglingia decumbens*, *Festuca ovina*.

HEATH FORMATION

- I. **Quercetum ericetosum.** Oak-birch association with *Q. robur*, *Q. sessiliflora*, *Betula alba*, *B. tomentosa*, *Fagus sylvatica* co-dominant, and *Pinus sylvestris* invading; also *Calluna*, *Vaccinium myrtillus*, and *Erica cinerea* amongst the oakwood ground flora.
- II. **Callunetum arenosum**, as for Upland Moor and Summit Heath, with *Ulex europæus*, *U. minor*, *U. gallii*, *Serratula*

tinctoria, *Veronica officinalis*, *Salix aurita*, *Schoenus nigricans* and *Lycopodium clavatum* as other species. In wet places *Myrica gale*, *Malaxis paludosa*, *Eleocharis multicaulis* and *Lycopodium inundatum* may occur with plants of the wetter upland moor associations.

- III. **Pinetum sylvestris.** Pinewood association, original or from I. by successful invasion, with *Pinus sylvestris* and *Calluna* dominant; *Trientalis europæa*, *Pyrola* spp., *Melampyrum pratense*, *Juniperus communis*, *Potentilla erecta*, *Oxalis*, *Hypericum pulchrum*, and plants of the upland moor. *Deschampsia*, *V. myrtillus*, and occasional mosses are the chief plants when the wood is dense.

FORMATION OF OLDER SILICEOUS SOILS

- I. **Quercetum sessilifloræ.** *Q. sessiliflora* with *Betula tomentosa*, also ash, alder, rowan, and elm; also many shrubs and herbs.
- II. **Siliceous Grassland Association**
- (a) **Nardetum strictæ**—with *Deschampsia flexuosa* or *Agrostis tenuis* associated; also *Ulex gallii*, *Pteris aquilina*, *Juncus* spp., and many other herbs.
- (b) **Molinietum cæruleæ.** *Molinia cærulea* with many plants of the wetter upland moors.
- III. **Betuletum tomentosæ.** Birchwood association with *Myrica gale* and plants of the oakwood and upland moor.

FORMATION OF CALCAREOUS SOILS

SUB-FORMATION OF OLDER LIMESTONES

- I. **Fraxinetum excelsioris.** Ashwood association, *Fraxinus excelsior* dominant; with wych elm, hawthorn, hazel, yew and juniper associated; sometimes also birch, rarely alder. *Allium ursinum*, *Asperula odorata*, *Hordeum sylvaticum*, *Nepeta hederacea*, *Lamium galeobdolon*, *Habenaria virescens*, and other herbs occur.
- II. **Limestone Scrub Association** includes blackthorn, *Euonymus europæus*, *Rhamnus catharticus*, *Cornus sanguinea*, *Helianthemum chamæcistus*, *Geranium sanguineum*, *Poterium sanguisorba*, *Saxifraga hypnoides*, *Inula squarrosa*, *Origanum vulgare*, *Listera ovata*.

Brachypodium gracile, *Koeleria gracilis*, and *Luzula pilosa*.

- III. **Festucetum ovinæ.** Limestone grassland association, with *Festuca ovina* dominant, includes *Anthoxanthum*, *Trisetum flavescens*, *Avena pratensis*, *A. pubescens*, *Arrhenatherum*, *Koeleria gracilis*, *Briza media*, *Bromus erectus*, *Brachypodium gracile*, and also herbs of the above scrub, together with *Anthyllis vulneraria*, *Hippocrepis comosa*, *Conopodium majus*, *Carlina vulgaris*, *Cnicus eriophorus*, *Gentiana amarella* and many orchids.

SUB-FORMATION OF THE CHALK

- IV. **Fagetum sylvaticæ calcareum.** Beechwood association of the chalk, with beech dominant, pure or with bird-cherry or yew; has a limited ground flora, including *Viola hirta*, *V. riviniana*, *Circæa lutetiana*, also dog's mercury, wood sanicle, wild strawberry and several orchids.
- V. **Fraxinetum.** Ashwood association similar to I.
- VI. **Festucetum ovinæ.** Chalk pasture similar to III., but with *Cynosurus cristatus*, *Dactylis glomerata*, *Bromus mollis* as additional grasses, and many other herbs.

SUB-FORMATION OF MARLS AND CALCAREOUS SANDSTONES

The associations here include mixtures from the "siliceous" and "calcareous" lists, e.g. for trees Ash and Oak to give an Ash-Oakwood Association with "damp oakwood" herbs and a few calcicoles, such as *Euonymus* and *Viburnum lantana*.

CHAPTER XXX

ENZYMES

505. Diastase. (a) Prepare a specimen of diastase (see p. 230).

(b) Prepare a solution of diastase; for methods see p. 230.

(c) Make a starch "solution" by shaking 0.1 gramme of starch with 10 c.c. water and pouring the mixture into about 100 c.c. of boiling water; cool. Mix in a test-tube 5 c.c. of the starch "solution" with 5 c.c. of diastase solution. Prepare eight similar tubes; boil one lot and test one of the others every fifteen minutes for starch by adding iodine solution to a 1 c.c. portion; also for sugar by adding a 1 c.c. portion to 1 c.c. of previously prepared Fehling's solution and boiling. Test, at the end, the contents of the boiled lot. Record your results systematically.¹

(d) Prepare an aqueous extract of malt and another of ungerminated barley (see. p. 230). Compare the diastatic powers of the two extracts by a series of tests as in (c) above, noting the relative times for complete conversion of the starch into sugar as indicated by the iodine test.

(e) Examine microscopically the starch grains of malt and of ungerminated barley. Correlate the evidence so obtained with the results given in (d) above.

506. Ptyalin. Compare the action of ptyalin on starch with the results of Ex. 505 (d).

507. Cytase. Examine microscopically thin sections from the middle parts of the germinating date-seeds supplied (see p. 230). Note the disintegration of the hemi-cellulose walls.

508. Invertase. (a) Shake about 1 gramme of yeast with 10 c.c. water. Set aside the mixture overnight in a warm place. Centrifuge repeatedly or filter through

¹ Purplish and red colours with iodine indicate the formation of intermediate products such as dextrans.

asbestos ; add 1 c.c. of the clear fluid to 1 c.c. of 5 per cent. solution of cane sugar. Set aside for two hours and test for reducing sugar. A negative result indicates that invertase is not an extracellular enzyme of yeast.

(b) Put 1 gramme of yeast in a mortar with 1 gramme of clean sand ; grind the mixture with a little water ; add ten drops of ether and continue grinding for ten minutes. Add 10 c.c. water ; mix well and obtain a clear fluid by filtration as before ; add 1 c.c. of the clear fluid to 1 c.c. of 5 per cent. cane sugar solution. Set aside for two hours, and test for reducing sugars. A positive result *now* indicates (1) that invertase is an intracellular enzyme of yeast, and (2) that invertase hydrolyses sucrose *in vitro*. For maltase see Ex. 515.

509. Inulase. Prepare an aqueous extract containing inulase (p. 230). Dissolve about 0.5 gramme of *pure* inulin in 50 c.c. hot water.

(a) Using 5 c.c. portions of the inulin solution make the following tests :—

- (i.) Add 1 c.c. inulase solution and five drops iodine solution—note absence of starch.
- (ii.) Add 1 c.c. previously boiled Fehling's solution and boil—note absence of reducing sugars.
- (iii.) Add 1 c.c. inulase solution and set aside for two hours in a warm place, see (b).
- (iv.) Boil 1 c.c. inulase solution for five minutes, then add this and set aside for two hours as above.

(b) Add 1 c.c. inulase solution to 5 c.c. water. Then prepare three tubes, A, B, C, each with 1 c.c. previously boiled Fehling's solution. Add to A 1 c.c. of diluted inulase solution ; to B 1 c.c. of the fluid from (iii.) ; to C 1 c.c. of the fluid from (iv.). Boil the three equally and compare by inspection the relative amounts of reduction. Record your results with an explanation of any differences noted.

510. Emulsin. Using 5 c.c. portions of a solution containing emulsin make the following tests :—

(a) Add 0.1 gramme of amygdalin¹ ; shake well ; and set aside for one hour in a quiet place. Note the odour of benzaldehyde.

(b) Boil well ; add 0.1 gramme of amygdalin and boil

¹ Amygdalin or amygdalose can be obtained commercially, together with other such materials, from the British Drug Houses and other firms.

again; fix picrate paper with a cork at the top of the tube, and set aside. Absence of reddening indicates destruction of the enzyme by boiling.

(c) Add 0.1 gramme of amygdalin and fix picrate paper with a cork as before. Rapid reddening indicates production of hydrocyanic acid; benzaldehyde is detected above (a); see Textbook, p. 413, also Ex. 330.

(d) Add 0.1 gramme of salicoside or salicin to each of two portions of emulsin solution. Boil the contents of one tube thoroughly and set both aside for two hours in a warm place. To each tube add 1 c.c. ferric chloride solution. Purple iron salicylate indicates hydrolysis in the unboiled tube.

511. Myrosin. The characteristic odour of allyl isothiocyanate, "mustard oil," may be used to detect the action of this enzyme. Soak 2 grammes of whole black mustard seeds in water overnight; also an equal quantity of white mustard seeds.

(a) Crush the black mustard in a mortar; triturate thoroughly with a little water. Note the odour. Add 10 c.c. water; mix well; transfer to a porcelain basin and boil until no pungent odour can be detected. Filter; the solution contains sinigroside with the myrosin destroyed; pour a half into each of two tubes.

(b) Crush the white mustard; and triturate with water until 10 c.c. have been used. Set aside in an open vessel overnight; filter. Note the odour; the solution now contains myrosin with some sinalboside. Divide the solution equally into two; boil one half; cool; add this to one of the tubes from (a) and the other half to the other tube from (a). Cork both lightly and set them aside for an hour. Note the difference in the odours. The unboiled myrosin again acts upon the sinigroside.

512. Proteases. Using 5 c.c. portions of a mixed protease solution, prepared as indicated on p. 231, make the following tests:—

(a) Using small pieces of white of egg and also of yolk investigate the action of the enzymes when the solution is (i.) previously well boiled; (ii.) previously acidified (*pH* 2.0) with dilute hydrochloric acid; (iii.) previously rendered slightly alkaline (*pH* 9–10) with dilute caustic soda solution. Digest all three tubes on a water-bath at 35° to 40° C. for two hours.

(b) **Pepsin Type.** Neutralise the acid digest from (a), (iii.) above. Filter off the precipitated **metaproteins**. Boil the filtrate, and while it is boiling acidify faintly to destroy the enzyme and coagulate the unchanged coagulable proteins. Filter. Test part of filtrate for **proteoses** with nitric acid (a precipitate dissolving on heating). Saturate the remainder of filtrate with ammonium sulphate. Filter off the precipitated **proteoses**. Test filtrate for **peptones** by the biuret reaction, adding solid sodium hydroxide and one drop of 1 per cent. solution of copper sulphate to get the pink or violet colour which indicates proteins, which, after precipitation of others with ammonium sulphate, should be only peptones.

(c) **Erepsin Type.** Allow alkaline digestion to proceed for several days, covering the top of the fluid with a thin layer of toluol as a preservative. Add a few drops of acetic acid; filter and (i.) test the precipitate, if any, for **tyrosin**; (ii.) test half the filtrate for **tryptophane** (see below); (iii.) neutralise the remainder with ammonia, evaporate to 0.5 c.c. or less, cool, and set aside. Crystals of **tyrosin** may form on standing.

Tests for Tyrosin. (i.) Add 0.5 c.c. water also a crystal of sodium chloride and a small quantity of dry quinone; warm. A ruby-red colour indicates tyrosin.

(ii.) Add concentrated sulphuric acid (0.5 c.c.) and two to three drops of 40 per cent. formaldehyde. Whenever the solution becomes red, add 1 c.c. glacial acetic acid, and warm carefully. A green colour indicates tyrosin.

(iii.) The fine colourless acicular crystals, often in clusters, with a high melting point (*ca.* 316° C.), are characteristic.

Test for Tryptophane. Using 1 or 2 c.c. of the solution add *bromine water*, one drop at a time, shaking after each drop. The characteristic pink or magenta colour given by this amino-acid is destroyed by excess of bromine. When it is obtained, the coloured substance can be separated by shaking gently with a few drops of amyl alcohol.

513. Oxidising Enzymes. Aqueous extracts containing *oxidase* give the following colour reactions: (1) blue with guaiacum solution (see Appendix II); (2) blue with benzidine solution¹; (3) violet with *α*-naphthol solution.¹ Aqueous extracts containing only the peroxidase element give the

¹ Benzidine and also *α*-naphthol are used as 1 per cent. solutions in 50 per cent. alcohol.

same colour reactions if a few drops of hydrogen peroxide are added after the test solutions.

(a) Demonstrate the occurrence of **oxidase** in one or more of the following—potato tuber, green cherries, plums, pears.

(b) Demonstrate the occurrence of **peroxidase** in one or more of the following : root of horseradish, turnip, radish ; fruit of cucumber, melon.

(c) Investigate the occurrence of oxidising enzymes in dandelion, hawthorn, forget-me-not (oxidases) ; and in pea, wallflower or other crucifers, violet (peroxidases).

(d) Investigate the occurrence of oxidising enzymes in bran, root-hairs, vascular bundles of various plant stems and petals of flowers.

514. Zymase. Mix in a test-tube 1 gramme of *zymin* (see p. 231) with 5 c.c. of 10 per cent. dextrose solution, and after at least twelve hours test for alcoholic fermentation as in Ex. 208.

515. Maltase. Mix in each of two test-tubes 0·2 gramme of *zymin* with 5 c.c. of 1 per cent. maltose solution ; boil one tube for ten minutes ; add to both a few drops of toluol and incubate at about 38° C. for forty-eight hours. Filter ; evaporate separately to about 1 c.c. and prepare the osazones as in Ex. 314. Compare under the microscope the maltosazone from the boiled tube with the glucosazone from the tube with the active enzyme.¹

516. Determine the nature of the enzyme in the 100 c.c. of solution supplied. Prepare six tubes as follows :—

(a) One cubic centimetre 0·1 per cent. starch solution ; (b) 1 c.c. 1 per cent. cane sugar solution ; (c) 1 c.c. 1 per cent. dextrose solution ; (d) 1 c.c. 1 per cent. inulin solution ; (e) 0·1 gramme dry amygdalin ; (f) 0·5 c.c. guaiacum solution.

To each add 5 c.c. of the enzymic solution ; set aside tubes (a), (b), (c), (d) ; fit picrate paper and a cork in tube (e) then set it aside ; if no blue colour has developed in (f) add a few drops of hydrogen peroxide. Positive results with (b) and (c) may indicate a mixed yeast extract, which might also react with (a) and (f). If all the results are negative maltase is possible, and proteases should be looked for, using pin-head portions of freshly coagulated white of egg.

¹ Much of this chapter depends upon Onslow's " Practical Plant Biochemistry," but the present writer apologises immediately for mentioning that precise and accurate manual in connection with the above rough approximations for less advanced students.

Confirmatory tests should be applied, for which see above, and also Onslow's "Practical Plant Biochemistry."

METHODS OF PREPARATION

Diastase

(a) Take 5 grammes of malt, or barley in the early stages of germination; pound this thoroughly in a mortar; add water in small quantities, triturating thoroughly after each addition, until 100 c.c. water have been used. Transfer the mixture to a beaker and set aside for three hours in a warm place, stirring occasionally. Filter.

(b) The extract obtained by the above method is placed in a round-bottomed flask attached to an active suction pump; heated gently on a water-bath not above 50° C. until concentrated to about 10 c.c. Alcohol (90 to 96 per cent.) is added in excess; the resulting precipitate is filtered, washed with 20 c.c. absolute alcohol and dried, if possible under reduced pressure, in a desiccator.

(c) A more convenient method of obtaining a solution containing diastase may be used if necessary. Dissolve a teaspoonful of commercial extract of malt in 100 c.c. of lukewarm water, not above 50° C.; this contains sugar.

(d) The dry impure enzyme is obtainable commercially and may be used in simple solution, 1 in 500 concentration.

Ptyalin. Mix saliva with an equal quantity of distilled water.

Cytase. Cover fresh date-stones completely with water in a beaker; keep these always immersed in water in an incubator between 60° C. and 70° C. for three weeks or until they have about 1 inch of germination tube.

Invertase. See Ex. 508 (b).

Inulase. Cut up and crush in a mortar two or three sprouting tubers of *Helianthus tuberosus*; transfer the mass to a wide-mouthed bottle; add sufficient water to cover the material and set aside in a warm place for twenty-four hours. Decant and filter some of the fluid.

Emulsin. Sweet almonds contain emulsin free from amygdalin; therefore they are preferred to bitter almonds for the preparation of this enzyme. Blanch half a dozen sweet almonds by removing the seed-coats with the aid of hot water; crush the kernels in a mortar; triturate with two successive 5 c.c. quantities of ether, rejecting the oily ether extracts. Add 50 c.c. water; triturate thoroughly;

transfer to a bottle; add one drop only of chloroform; shake well and set aside for twenty-four hours. Decant and filter.

Myrosin. See Ex. 511.

Proteases. (a) Pepsin and trypsin (erepsin type), the latter also as *Liquor Pancreatis*, are both obtainable commercially in the dry condition.

(b) A mixture of enzymes of these two types can be obtained by aqueous maceration of figs at temperatures below 50° C.

(c) Commercial papain includes both types from a plant source.

(d) Both occur also in hemp seed, from which they can be extracted thus: Soak 10 grammes of hemp seed in water overnight; grind them in a mortar, with sand if necessary; add 50 c.c. 10 per cent. sodium-chloride solution in small quantities, triturating thoroughly after each addition. Set aside overnight in a cool place; filter.

Oxidising Enzymes. (a) Fresh aqueous extracts of the various tissues are prepared rapidly by cutting the material into small, thin pieces, grinding this in a mortar with twice its weight of water and filtering.

(b) Sections may be mounted and examined under the microscope when the distribution of enzyme is localised as in some petals.

Zymase. Extract 10 grammes of yeast with two successive quantities of 20 c.c. acetone on a small Buchner funnel lined with three layers of filter paper; dry by suction filtration; powder in a mortar; add just sufficient ether to make a workable paste; triturate for five minutes; spread out on filter paper to dry in a safe place away from any naked flame. This dried dead yeast powder is known as *zymin*, and can be used as an extracellular mixture of enzymes which includes zymase and maltase.

N.B. Advanced students will find many other experiments and much valuable information concerning enzymes, their distribution and reactions, in "Practical Plant Biochemistry," by M. W. Onslow. Cambridge University Press.

PART II

CHAPTER XXXI

PRINCIPLES OF CLASSIFICATION

517. THE recognition of similarities and differences, together with the grouping of degrees of similarity and the separation of degrees of difference, is the fundamental principle of classification. Even the first-year student should carry out at least one exercise involving this principle.

(a) Classify and give an account of the various shapes of ivy leaves which can be found locally.

(b) Given a mixed collection of (i.) grass inflorescences, or (ii.) grass leaves, classify them and give an account of your methods of grouping.

(c) Similar exercises can be carried out on mixtures of seeds or small fruits.

518. Prepare diagrams (see Fig. T 901) showing the status and names of the various sub-divisions of the plant kingdom for the following : *Spirogyra*, *Agaricus*, *Funaria*, *Aspidium*, *Pinus*, *Cycas*, *Rosa*, *Ferula*, *Erica*, *Primula*, *Solanum*, *Taraxacum*.

519. Prepare a diagram to show the possible relationships of the following : *Chlamydomonas*, *Saccharomyces*, *Laminaria*, *Riccia*, *Polytrichum*, *Selaginella*, *Ephedra*, *Ranunculus*, *Rosa*, *Scabiosa*, *Lobelia*, *Senecio*, *Taraxacum*, *Carduus*.

520. By reference to appropriate books give a comparative account of the principles of classification used for flowering plants by Theophrastus, *e.g.* in his "Enquiry into Plants," by Linnæus in his "artificial system," which is to be found in many old books, and by De Candolle in his "Prodromus," which is the basis of modern systems. Follow at least three examples through the three stages.

521. Write brief notes on the principles of classification which lead to the following distributions :

(a) *Caltha palustris* and *Ranunculus acris* are in the same family, while *Spiræa* and *Astilbe* are in different families.

(b) *Geranium*, *Tropæolum* and *Limnanthes* are by some authorities placed in three different families and by others all in the same family.

(c) *Cycas*, *Pinus* and *Welwitschia* are all in the Gymnospermae, but in different divisions.

CHAPTER XXXII

THE PHYLA

BEFORE commencing the detailed study of the lower plants the first-year student should make practical comparisons which involve the main distinguishing characters of the phyla.

522. Compare under the microscope the spores of *Lycopodium* with minute seeds, e.g. of an orchid, or *Calceolaria* or Iceland poppy.

523. Compare and draw under H.P. prepared specimens of the oogonium of *Vaucheria* or other alga, and the archegonia of a liverwort, a moss, a fern, and *Pinus*.

524. Compare and make annotated drawings of the thallus of *Vaucheria*, *Fucus* or *Laminaria*, *Agaricus*; the fruiting plants of *Pellia* or *Marchantia*; *Sphagnum* or *Funaria* or other moss; the prothallus and sporophyte of a fern, the prothallus and seedling of *Pinus*, also the embryo sac and plant of daffodil or wallflower. Label the following where they occur: thallus, rhizoids, stem, roots, gametophytic leaves, sporophytic leaves, sporophyte and gametophyte.

CHAPTER XXXIII

ALGÆ

First Year Types

525. *Chlamydomonas*. A motile unicellular green alga. Place a drop of water, containing living specimens, on a slide, and lower the cover-slip gently. Under low-power note numerous green cells moving actively. Find one which has come to rest and examine under high power. Note and draw *on a large scale*: (a) oval cell wall; (b) large cup-shaped green chloroplast which lies in the cytoplasm and occupies almost the whole cell; (c) within the base of the chloroplast lies a pyrenoid, consisting of nitrogenous substance, which may be surrounded by starch granules; (d) colourless cytoplasm at anterior end of cell from which 2 cilia arise (if cilia cannot be seen irrigate with iodine solution after having drawn the specimen); (e) a small red pigment spot may be seen. The nucleus is usually concealed by the chloroplast and is difficult to see (Fig. T 913).

526. *Vaucheria*. Mount a few filaments carefully in water. The whole thallus is one large multinucleate cell or cœnocyte, with many branches. Note and draw appearance under low-power. Under high-power note and draw (a) external cell wall (there are no cross-walls in the vegetative filaments); (b) very numerous small chloroplasts which are embedded in the lining layer of cytoplasm; (c) numerous refractive oil drops in the cytoplasm (reserve food material). Many nuclei are present, but these cannot be seen without special staining (Fig. T 925).

Reproductive Organs. Under H.P. examine and draw: (a) antheridium, a short curved branch the end of which is cut off by a transverse wall; the contents form motile antherozoids (male gametes); (b) oogonium, nearly globular in shape; note transverse wall at the base. The contents form a single large non-motile oosphere or egg cell (female gamete). In some cases a fertilised egg cell (oospore) may

be seen. This can be distinguished by the thick cell wall (Fig. T 924).

527. *Spirogyra*. A multicellular green alga consisting of unbranched filaments of cells placed end to end. Mount in water; note and draw two or three cells to show: (a) spiral chloroplasts in which pyrenoids are embedded; (b) nucleus. Some filaments may show stages in conjugation. Make drawings to show different stages in conjugation. (Figs. T 922–923).

528. *Fucus vesiculosus* or *F. serratus*. Examine and draw thallus of *Fucus*. Note attachment disc, stalk, "fronds," dichotomous branching, growing points. The extreme apex is sunk in a depression. Observe fertile branches with conceptacles. Male and female conceptacles are on different plants. Examine demonstration sections through male conceptacles. Note and draw (1) flask-shaped cavity with an ostiole; (2) colourless branched hairs bearing brownish oval antheridia; (3) sterile hairs. Examine demonstration sections through female conceptacles. Note greenish oogonia—large oval bodies some of which may have undergone division into a number of egg cells. Eight egg cells are formed (Figs. T 937–942).

Second Year Types

N.B. (1) For classification see Textbook, pp. 456–458, also "British Fresh-water Algæ," by West and Fritsch (Cambridge University Press).

(2) Drawings should be made of all observed general forms and structural details.

CYANOPHYCEÆ

Group I. Chroococcales—unicellular or in loose indefinite groups.

Glæocapsa. Examine squash preparations and draw a few groups. This occurs as gelatinous yellowish masses on old wood in or about heated glass-houses.

Group III. Hormogoneales—filaments with or without heterocysts.

Nostoc. Note gelatinous mass, embedded in which are chains of rounded cells with intercalary or terminal large colourless heterocysts, each with slight thickenings where the hormogones are attached.

Collema. Note the *Nostoc* filaments, also the fine mycelial threads of the associated fungus; *Collema* is a dark green gelatinous lichen.

Anabaena. In a transverse section of *Cycas* root, note the

endophytic *Anabaena cycadearum*, very similar to *Nostoc* (see Ex. 217). Similar endophytic filaments are to be found within the leaves of *Azolla*.

Oscillatoria. Note faint septation and sheath, also distinct wall and rounded ends of sections or hormogones.

CHLOROPHYCEÆ

Class I. Isokontæ

Group I. Volvocales

Series I. Chlamydomonadales

Fam. 2. Chlamydomonadaceæ. *Chlamydomonas*; examine fresh material (see Fig. T 913 and Ex. 525).

Fam. 3. Sphærellaceæ. *Volvox*; examine fresh cœnobia; look for young cœnobia within parent; also androgonidia (16-64) and gynogonidia (4-8) (see Fig. T 926).

Series III. Tetrasporales

Fam. 1. Palmellaceæ. *Palmella* (see Fig. T 913).

Group 2. Chlorococcales

Series I. Zoosporinæ

Fam. 3. Hydrodictyaceæ. *Pediastrum*; a single layer of small cœnocytes united to form a free-floating cœnobium (see prepared slide, also Fig. T 927).

Group 3. Ulotrichales

Series I. Eu-Ulotrichales

Fam. 1. Ulotrichaceæ. *Ulothrix*; examine fresh material and mount in glycerine jelly (see Figs. T 916-918).

Fam. 3. Ulvaceæ. *Ulva*; mount a portion or section; note two cells thick, each cell with one parietal chloroplast and one pyrenoid.

Enteromorpha; mount a portion; note single tubular layer of cells, enclosing a watery cavity. Compare with *Ulva*.

Series III. Cladophorales

Cladophoraceæ. *Cladophora*; examine fresh material. Note wall formation by ingrowth, also reticulate chloroplast with large pyrenoids. Stain a portion in hæmatoxylin (10-15 minutes), pass through alcohol, absolute alcohol, erythrosin in clove oil, to xylol; mount in Canada balsam.

Group 4. Chætophorales

Fam. 2. Trentepohliaceæ. *Trentepohlia*; orange red mat on rocks or tree-trunks. Note hæmatochrome in sap, numerous chloroplasts, terminal or lateral sporangia, apical thickening of wall in end cells.

Fam. 3. Coleochætaceæ. *Coleochæte*; examine prepared

slide ; note meristem and special reproduction (see Textbook, pp. 445–459, also Fritsch and West, p. 202).

Fam. 5. Pleurococcaceæ. *Pleurococcus* ; examine fresh material (see Figs. T 914–915).

Group 5. Oedogoniales

Fam. Oedogoniaceæ. *Oedogonium* ; examine fresh or preserved material (see Figs. T 919–921).

Group 6. Conjugatæ

Series I. Eu-Conjugatæ

(b) Zygnemales.

Fam. 2. Zygnemaceæ. *Spirogyra* ; examine fresh or preserved material in detail (see Ex. 527 and Figs. T 922–923).

Fam. 3. Mougeotiaceæ. *Mougeotia* (see Ex. 406).

Series II. Desmidiaceæ. *Closterium*, etc.

Group 7. Siphonales

Fam. 1. Vaucheriaceæ. *Vaucheria* (see Ex. 526 and Figs. T 924–925).

Fam. 2. Caulerpaceæ. *Caulerpa* ; examine museum specimen and prepared slides ; large cœnocyte, note transverse trabeculæ (see Fig. 929).

Fam. 3. Codiaceæ. *Codium tomentosum* ; examine material and T.S. ; thallus much branched, cœnocytic ; a marine type with pseudo-parenchyma, rhizoids and many small parietal chloroplasts.

Class II. Heterokontæ

Group 4. Heterosiphonales

Fam. Botrydiaceæ. *Botrydium* ; examine prepared slide (see Fig. T 928).

Class IV. Bacillariales (Diatomales)

Examine and draw prepared material of the following (see Fig. T 936 A).

Group A. Centricæ

Series I. Discoideæ. *Melosira*, *Cyclotella*.

Group B. Pennatæ

Series I. Fragilarioideæ. *Tabellaria*, *Meridion*, *Licmophora*, *Diatoma*.

Series III. Naviculoideæ. *Navicula*, *Gomphonema*.

PHÆOPHYCEÆ

Phæosporales

Ectocarpaceæ (Rabenhorst) includes *Pylaiella* and *Cladostephus*, p. 241.

Ectocarpus; examine mounted specimens. *E. siliculosus*; examine material and prepared slides of zoosporangia (see Figs. T 945-946).

Mesoglraceæ includes *Chordaria*, *Elachista* and *Leathesia*.

Punctariaceæ. *Desmarestia aculeata*; mount an apical portion, also T.S. thallus low down (see Fig. T 943).

Cutleriaceæ. *Cutleria*; examine prepared slides and herbarium specimens (see Fig. T 946).

Cyclosporales

Fucaceæ. *Fucus*; examine material in detail (see Ex. 525 and Figs. T 937-942). Note habit of *F. vesiculosus*, *F. serratus*, *F. spiralis*.

Ascophyllum nodosum; note general habit, with *Polysiphonia* epiphytic.

Sargassum; examine herbarium specimens (see Fig. T 946).

Pelvetia canaliculata; examine material; note monœcious conceptacle (prepared slide), also limiting layer and apical cell (see Figs. T 947-948).

Himanthalia lorea (see Fig. T 946) note general habit.

Laminariaceæ includes *Chorda* and *Alaria*, p. 240-241.

Laminaria; note general habit of *L. saccharina* also *L. digitata* with hapteron, stipe and lamina; cut T.S. and L.S. stipe (see Fig. T 944). *Laminaria* reproduces by means of zoospores produced in sporangia situated in a furrow at the base of the lamina. These zoospores settle to form microscopic unisexual gametophytes, which develop in various ways (see Lloyd Williams in *Ann. Bot.*, 35, 121).

Dictyotaceæ. *Dictyota dichotoma*; examine herbarium specimens; also material; stain in eosin and mount an apical portion; also sori and tetrasporangia; examine prepared slide of T.S. thallus (see Fig. T 940).

Padina pavonia; note general habit in herbarium specimen.

RHODOPHYCEÆ

I. Bangiales includes *Bangia* and *Porphyra*.

II. Florideæ includes the following:—

Nemalionales

Nemalion; examine material and prepared slide (see Figs. T 952-953).

Gelidium; note herbarium specimens, also specimens of agar-agar.

Gigartinales

Chondrus crispus; examine material and herbarium specimens (see Fig. T 902).

Gigartina mamillosa ; examine material ; note mammillæ.

Rhodymeniales

Polysiphonia fastigiata ; examine material in detail ; note thallus in T.S. and L.S., also tetraspores, antheridia, cystocarps, etc. (see prepared slides and Figs. T 946, T 950).

Ceramium ; examine prepared slides, also material (see Fig. T 951).

Plocamium coccineum ; examine mounted specimens, also prepared slide (Fig. T 954).

Lejolisia ; note prepared slides (Fig. T 953-954).

Cryptonemiales

Dudresnaya ; examine prepared material.

Corallina ; note general habit in mounted specimens.

Note :

Ceramiceæ—includes *Rhodochorton*, *Callithamnion*, *Griffithsia*, *Ptilota*, *Dudresnaya* and *Ceramium*, see below.

Cryptonemiaceæ—includes *Dumontia*, *Halymenia*, *Furcellaria* and *Iridaea*.

Gigartineæ—includes *Chondrus* and *Gigartina*.

Rhodomeneæ—includes *Chylocladia*, *Rhodymenia*, *Plocamium*.

Rhodomelaceæ—includes *Laurencia* and *Polysiphonia*.

COASTAL ZONATION

The following brief summary of a more or less typical flora in the tidal zone of a sloping rocky shore has proved helpful as a general guide to beginners ; many variations occur and many of the forms in the lowest zone are to be found in rock-pools at higher levels.

Lichens—*Ramalina scopulorum*, *Xanthoria parietina*.

Verrucaria maura (black), *V. mucosa* (greenish), *Lichina confinis*, *L. pygmaea*.

Spring Tide H.W.M.—

Pelvetia canaliculata, *Cladophora rupestris*.

Neap Tide H.W.M.—

Fucus spiralis or *Fucus platycarpus*, *Ulva latissima*, *Enteromorpha intestinalis*, *Porphyra laciniata*, *P. vulgaris*.

Ascophyllum nodosum with *Polysiphonia fastigiata* epiphytic and many shade forms of lower zones below, e.g.

Lomentaria (*Chylocladia*), *Ceramium*, *Plocamium*, etc.

Fucus vesiculosus.

Neap Tide L.W.M.—

Fucus serratus.

Halidrys siliquosa, *Alaria esculenta* (small), *Himantalia lorea*, *Laurencia pinnatifida* or *L. caespitosa*, *Gigartina mamillosa*.

Laminaria digitata.

Laminaria saccharina.

Alaria esculenta (large).

Spring Tide L.W.M.—

Chondrus crispus, *Callithammion* spp., *Corallina officinalis*, *Ceramium rubrum*, *Dumontia filiformis*, *Delesseria alata*, *D. sanguinea*, *D. sinuosa*, *D. sericea*, *Gigartina mamillosa*, *Ptilota plumosa*, *Plumaria elegans*, *Chylocladia* (*Lomentaria*) *articulata*, *Plocamium coccineum*, *Laurencia* spp., *Rhodymenia laciniata*, *R. palmata*, *Iridaea edulis*, *Furcellaria fastigiata*, *Griffithsia setacea*, etc.; also brown algæ, *Desmarestia aculeata*, *Ectocarpus* spp., *Asperococcus*, *Chorda filum*, *Chordaria*, *Dictyota dichotoma*, *Cladostephus spongiosus*.

Algæ of Sand and Sandy Mud include *Enteromorpha intestinalis*,¹ *Rhizoclonium* sp., *Rhodochorton floridulum*, *R. rothii*. *Bangia* spp. may occur on rock surfaces.

¹ *E. compressa* occurs in the mud-flats of the salt-marsh formation, also on flat sandy beaches, especially in the neighbourhood of fresh-water streams.

CHAPTER XXXIV

FUNGI

First Year Types

529. *Mucor*. A saprophytic mould fungus. Note dense felt of mycelium, which is made up of colourless threads of hyphæ growing on decaying organic matter. Observe sporangiophores, each bearing a spherical sporangium at tip. Mount in water; under H.P. note and draw: (a) cell wall; (b) granular cytoplasm; (c) sporangium filled with spores; (d) columella—a cross-wall projecting into the sporangium. Examine demonstration slide showing zygospores of *Rhizopus*—a fungus closely allied to *Mucor* (Figs. 958–959).

530. *Cystopus*. A fungus growing parasitically on *Capsella*. Examine material of *Capsella* infected with *Cystopus*; notice the white patches on the stem; some will be seen covered with the epidermis and in other cases the epidermis will be broken. Examine given T.S. stem of infected *Capsella*. Note and draw (1) colourless non-septate hyphæ in intercellular spaces; (2) haustoria—absorbing organs of the fungus, which penetrate into the cells of the host plant; (3) under the epidermis the fungus as seen in the conidial stage. The epidermis is separated from the underlying tissue by a mass of hyphæ, each hypha ending in a chain of conidia formed by abstriction. Examine demonstration slides of *Cystopus* showing oospheres (Figs. 960–961).

531. *Saccharomyces* (yeast fungus). Examine drop of yeast culture. Observe under H.P.: (a) cell wall; (b) cytoplasm; (c) nucleus; (d) glycogen. Note mode of vegetative multiplication of the organism by budding. Look for chains of cells (Figs. T 899A, 984–985).

Second Year Types

N.B. (1) For classification and details, see Textbook, pp. 459–480, also “The Structure and Development of the Fungi,” by Gwynne-Vaughan and Barnes (Cambridge University Press).

(2) Drawings should be made of all observed general forms and structural details.

MYXOMYCETES

1. Myxogasterales (includes most British myxomycetes)

Badhamia utricularis—habitat higher fungi; examine plasmodium, sporangia (see prepared slides), also sclerotium (see Figs. T 955–956).

The sporangial condition of many of these forms dries well, and students should see a selection of prepared specimens, such as species of *Arcyria*, *Trichia*, *Fuligo*, *Stemonites*, *Comatricha*, *Craterium*, *Didymium*, *Lycogala*, *Physarum*, etc.; for details, see "Guide to the British Myxomycetes," British Museum.

2. Phytomyxales

Plasmodiophora brassicæ (Finger and Toe Disease); examine and draw infected turnip root; mount and examine the section supplied (see Fig. 957).

Spongospora subterranea (Corky Scab of Potato); examine infected tuber, also a section through a scab; note spore-balls and look for internal cork layer localising infection; this may be absent.

PHYCOMYCETES

I. Archimycetes

Chytridiales

Synchytriaceæ. *Synchytrium endobioticum* (Black Scab of Potato); examine prepared specimens and prepared slide of resting sporangium; also prepared slide of *S. taraxaci*.

Protomycetales

Protomycetaceæ. *Protomyces macrosporus*; note blisters on bishopweed leaf-stalks and rhizomes; examine prepared slide of resting sporangium.

II. Oomycetes

Saprolegniales

Saprolegniaceæ. *Saprolegnia*; material on immersed flies, mount in water and examine for zoosporangium, oogonium with several oospheres, antheridium.

Achlya; note prepared slides of zoosporangia and oogonia (see Figs. T 966–967).

Peronosporales

Pythiaceæ. *Pythium debaryanum*; examine infected cress seedlings; external conidia and zoosporangia; internal oogonia with or without antheridia (see Fig. T 962).

Phytophthora infestans (Potato Blight); cut T.S. infected leaf; note conidiophores, conidia, intercellular hyphæ and intracellular haustoria (see Figs. T 963–964). The haustoria are usually less narrow and less branched

than in Fig. T 963. Sexual reproduction has been described; the oogonium sends a tube completely through the antheridium, and this tube then swells to form an oospore on the far side of the male organ.

Albuginaceæ. *Cystopus candidus* (*Albugo candida*); examine infected *Capsella* plants externally, and in T.S. note intercellular hyphæ and intracellular haustoria, also prepared slide of oogonia and antheridia (see Figs. T 960–961).

Peronosporaceæ; includes *Plasmopara viticola* (see Fig. T 965); note *Peronospora ficariæ* on lesser celandine.

III. Zygomycetes

Mucorales

Mucor mucedo (Black Mould)¹; examine material; note mycelium, sporangiophore, sporangium with columella (see Fig. T 958 and Ex. 529).

Mucor stolonifer (*Rhizopus nigricans*); heterothallic, note prepared slides of stages in conjugation (cp. Fig. T 959).

Sporodinia grandis; homothallic, note prepared slide of zygospores.

ASCOMYCETES

I. Plectomycetes

Plectascales

Saccharomycetaceæ. *Saccharomyces*; examine active fresh material in detail (see Figs. T 899A, also T 984 985).

Aspergillaceæ. *Aspergillus* (*Eurotium glaucum*); note in prepared slides ascogonium (oogonium), antheridium, ascocarp, asci; also conidia and sterigmata (see Fig. T 969–971).

Penicillium; mount material in glycerol; note conidiophores, conidia; also prepared slide of ascocarp (see Fig. T 972).

Erysiphales

Erysiphaceæ. *Sphaerotheca mors-uvæ*; examine material; note conidial stage and perithecial stage in detail (see Fig. T 968).

Erysiphe graminis; examine prepared slides of conidia and ascocarps.

II. Discomycetes

Pezizales

Pyronemaceæ. *Pyronema*; note prepared slide of oogonia (oogonia) and antheridia.

¹ Grown on wet bread which may require infection with laboratory dust.

Pezizaceæ. *Peziza* ; cut a vertical section of apothecium ; stain in aqueous eosin ; mount and note peridium, pseudoparenchyma, hymenium with asci and paraphyses (see Figs. T 973–976).

Helvellales includes *Helvella* and *Geoglossum*.

Tuberales includes *Tuber* (Truffle).

Phacidiales : **Phacidiaceæ** : *Rhytisma acerinum*—note black circular sclerotium on leaves of *Acer*, also in spring the apothecium below.

III. Pyrenomycetes

Hypocreales

Nectriaceæ. *Nectria cinnabarina* (coral spot) ; examine material, also prepared slides of conidial and perithecial stages of stromata. *N. galligena* (apple canker) develops perithecia on withered apples.

Claviceps purpurea ; examine prepared specimens, also prepared slides and note stromata, perithecia, asci and spores (see Figs. T 903, also T 977–982).

Cordyceps ; examine prepared specimens (see Fig. T. 983).

Sphaeriales includes *Chaetomium* found on damp wallpaper, *Sordaria* and *Xylaria* (Candle-tuft).

BASIDIOMYCETES

I. Hemibasidiomycetes

Ustilaginales

Ustilaginaceæ (basidia septate) *Ustilago carbo* ; examine infected oats (see Fig. 986).

Tilletiaceæ (basidia continuous) *Tilletia tritici* ; examine prepared slide of germinating teleutospore.

II. Protobasidiomycetes

Uredinales

Pucciniaceæ—

Puccinia graminis ; heterœcious ; examine prepared slides of all stages (see Figs. 987–990).

P. poarum ; cut V.S. of œcidiosori on coltsfoot leaf.

P. pringsheimii ; examine œcidial stage on gooseberry leaf.

P. malvacearum ; examine teleutospore on hollyhock leaf.

Phragmidium violaceum ; autœcious ; note all spore forms on blackberry (*Rubus*), teleutosori and uredosori on leaf.

Auriculariales—*Hirneola auricula-judae*—note prepared specimen.

Tremellales includes *Tremella mesenterica* (witch's butter) and *Tremellodon*.

III. **Autobasidiomycetes**(a) **Hymenomycetales**

Thelephoraceæ—includes *Stereum purpureum* and *Corticium*.

Clavariaceæ—includes *Clavaria*, *Typhula* and sometimes *Dacryomyces*.

Hydnaceæ—includes *Phlebia* and *Hydnum*.

Agaricaceæ—includes many larger fungi, see “Handbook of the Larger British Fungi,” by J. Ramsbottom, British Museum.

Agaricus; examine material; note stipe, pileus, lamellæ and annulus. Cut V.S. lamellæ and L.S. stipe; mount and examine basidia, sterigmata and basidiospores (see Figs. T 991–993).

Polyporaceæ—includes *Merulius lachrymans* (dry rot), *Polyporus*, *Polystictus*, *Boletus*, etc.

(b) **Gasteromycetales** includes *Phallus*, *Clathrus*, *Lycoperdon*, *Geaster*, *Nidularia*, *Crucibulum*, *Cyathus*; note prepared specimens.

CHAPTER XXXV

LICHENES

First Year Types

532. NOTE museum specimens of crustaceous, foliaceous and fruticose types ; including *Xanthoria*, *Peltigera*, *Cetraria islandica*, *Usnea* and *Cladonia* (cp. Figs. T 996-997, also T 904).

Second Year Types

N.B. (1) For classification and details see Textbook, pp. 481-486, also "Lichens," by A. L. Smith, Camb. Univ. Press ; "Classification of the Lichens," by W. Watson, C.U.P. ; or "Nouvelle Flore des Lichens," by A. Boistel, Lib. Gén., Paris.

(2) Drawings should be made of all observed general forms and structural details.

ASCOLICHENES

I. Pyrenocarpeæ

Verrucaria nigra ; note prepared specimens.

V. maura ; see on rocks near H.W.M., p. 240.

II. Gymnocarpeæ

Coniocarpineæ—note prepared specimen *Coniocarpon*.

Graphidineæ—note prepared specimen of *Roccella tinctoria* and *Graphis* spp.

Cyclocarpineæ

Peltigera canina ; foliaceous ; examine V.S. thallus ; note apothecia and rhizines (cp. Fig. T 999).

Xanthoria (Parmelia) parietina ; crustaceous ; examine specimens, also prepared slide of V.S. thallus and apothecium ; draw this under H.P. and label upper cortex, algal zone, medulla, lower cortex, rhizines, asci and ascospores (cp. Figs. T 999 and T 974).

Cladonia ; examine podetia under pocket-lens ;

LICHENES

mount and examine under H.P. soredia from interior of cup; cut T.S. podetium. Note also museum specimens of all other forms mentioned in the Textbook, p. 486.

BASIDIOLICHENES

Cora pavonia; note and draw prepared specimens (see Fig. T 998).

CHAPTER XXXVI

BACTERIA

First Year Types

533. EXAMINE a drop of hay infusion in which decaying organic matter is present. Under L.P. note numerous very minute bacteria. They are motile by means of very delicate cilia, visible only after special staining. Under H.P. observe shapes of bacteria. Most of them will be *Bacillus subtilis* which is rod-shaped (*bacillus form*), but others may be present, e.g. spherical cells (*coccus form*) or spiral cells (*spirillum form*).

534. Scrape the surface of one of your teeth with a pin and mount the deposit in a drop of water. Examine under H.P. and look for the following: (a) *Bacillus buccalis*—thick thread-like filaments; (b) *Leptothrix buccalis*—thin thread-like filaments; (c) *Spirochaete dentium*—motile spiral cells; (d) *Spirillum sputigenum*—motile comma-shaped rodlets; (e) *Micrococcus*—small spherical cells. After examining the bacteria in water, irrigate with iodine. *B. buccalis* will probably stain blue, showing presence of starch, while both it and *Leptothrix* may now be seen to consist of a number of cells placed end to end (cp. Figs. T 1005–1006).

Second Year Types

N.B. (1) For classification and details see below, also "Determinative Bacteriology," by D. H. Bergey (Baillière, Tindall and Cox). For details of technique, see Part IV "Mycology and Plant Pathology," by J. W. Harshberger (Churchill).

(2) Drawings should be made of all observed general forms of colonies and structural details.

Cleaning and Sterilisation. Glassware should be washed with soap and water, and dried in an incubator or water-oven. Inoculation needles, forceps, scalpels, and cover-slips may be sterilised by boiling in water or by passing three times through a Bunsen flame. Prepared tubes or flasks of media should be plugged tightly with cotton-wool and sterilised in an autoclave at one and a half atmospheres of pressure for at least fifteen minutes.

General Investigations

(1) Prepare a stale infusion of hay (see Ex. 533), broad beans, peas or other dry vegetable material; inoculate a plate (Petri capsule) of agar¹ and 0.2 per cent. dextrose from the infusion; and note the characters of the resulting colonies.

(2) Extend Ex. 534 to a study of the forms present as grown on a neutral agar-dextrose medium.

(3) Prepare tubes of agar-dextrose with a sloping surface and inoculate the surface from the buccal cavity by means of a sterile needle placed in the mouth and then drawn across the sloping surface of the medium to get a streak culture.

(4) Expose sterile plates of agar-medium to which has been added an equal quantity of aqueous 1 per cent. peptone (a) to the air of the laboratory, and (b) to the air outside, each for only ten minutes. Incubate the closed capsules at 25° C. and note the number and other characters of the resulting colonies.

In the above exercises the following characters should be noted: number of colonies; rate of growth; form, including elevation, edge and internal structure; colour, liquefaction; form of cells; method of grouping, *e.g.* in chains, etc.; formation of endospores; cilia, capsulating membranes, zooglyca; staining reactions with carbol fuchsin¹ and methylene blue.¹

Classification of Bacteria

The following much abbreviated classification is derived from the third edition of Bergey's "Manual of Determinative Bacteriology."

Order I. Eubacteriales—more or less spherical, with or without endospores.

Fam. 1. Nitrobacteriaceæ—chemosynthetic; includes *Nitrosomonas*, *Nitrobacter*, *Azotobacter*, *Rhizobium* (= *Pseudomonas*).

2. Cocceæ—cocci, no endospores; includes *Diplococcus*, *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Sarcina*, *Rhodococcus*.

3. Spirillaceæ—commas or spirals, not flexuous; includes *Vibrio* and *Spirillum*.

4. Bacteriaceæ—saprophytes or parasites, no endospores; includes *Serratia marcescens* (= *Bacillus prodigiosus*) (red), *Flavobacterium* (yellow), *Chromobacterium* (violet), *Pseudomonas* (greenish), *Eberthella typhi* (= *B. typhosus*).

5. Bacillaceæ—saprophytes or parasites, endospores formed; includes *Bacillus subtilis* and *Clostridium* (*C. tetani* = *B. tetani*).

Order II. Actinomycetales—filamentous, conidia may be formed; includes *Actinomyces*.

Order III. Chlamydobacteriales—alga-like, conidia may be formed;

¹ See Appendix II.

includes *Leptothrix ochracea*, also *Didymohelia ferruginea* (= *Spirillum ferrugineum*).

Order IV. Thiobacterales—sulphur granules stored, no conidia; includes *Thiothrix* and *Beggiatoa*.

Order V. Myxobacterales—pseudo-plasmodium, cysts, but no conidia and no endospores formed.

Order VI. Spirochætales—flexuous spiral cells; includes *Spirochæte*.

CHAPTER XXXVII

BRYOPHYTA

First Year Types

535. LIVERWORTS. Examine and draw external features of *Pellia*. I. *Gametophyte*. Note flattened green thallus, dichotomously branched, and brownish rhizoids on lower surface. The antheridia occur on the upper surface of the thallus and may be seen with the naked eye as dots. The archegonia arise in groups behind the growing points. Examine prepared slides showing antheridia and archegonia. Make careful drawings to show details. II. *Sporophyte*. Examine and draw thallus (gametophyte) of *Pellia* with sporogonium (sporophyte) attached. Examine also prepared slide showing young sporogonium (Fig. T 1014).

536. MOSSES. Examine and draw external features of *Sphagnum* (Bog-moss). I. *Gametophyte*. Note leafy plant made up of erect stem and thin leaves. Stem is branched. Some branches grow erect, others turn down and are adpressed to the axis. Mount a leaf of *Sphagnum*. Note large water-storing cells and small chlorophyll-containing cells. Sexual organs are borne on special branches of the gametophyte, antheridia on one branch and archegonia on another. Examine material with antheridia. Also examine and draw prepared slides of antheridia and archegonia of a moss. II. *Sporophyte*. The sporogonium itself has no stalk but it is raised up above the leaves on an outgrowth of the gametophyte and it remains attached to this by a foot. Dissect out a sporogonium of *Sphagnum*; note columella and bell-shaped spore sac containing spores (Figs. T 1018–1022). Draw details of *Sphagnum*, gametophyte and sporophyte.

537. Examine and draw in detail the gametophyte and sporophyte of *Funaria* (see Textbook figures, T 1028–1036).

Second Year Types

N.B. (1) For classification see Textbook, p. 510; also "Handbook of British Hepatics," by S. M. MacVicar; "Handbook of

British Mosses," by H. N. Dixon, both published by V. V. Sumfield, Eastbourne; "Nouvelle Flore des Mousses et des Hépatiques," by M. Douin (Lib. Gén., Paris).

(2) Drawings should be made of all observed general forms and structural details.

Hepaticæ

I. Marchantiales

Ricciaceæ—*Riccia fluitans*; note in fresh or preserved material—dichotomy, midrib, growing point; examine prepared slide of V.S. thallus and sporogonium (*R. glauca* or other species) (cp. Fig. T 1007).

Marchantiaceæ—*Marchantia polymorpha*; note in fresh or preserved material—circular gemmæ cups, barrel-shaped pores, rhizoids, amphigastria; prepared material of antheridiophores and archegoniophores; also prepared sections of these and of young sporogonia; note the grooves in the stalks. Cut and mount a vertical section of the thallus; label all details such as assimilating filaments, smooth and tuberculate rhizoids, amphigastria, etc. (see Figs. T 1008–1013).

Lunularia cruciata; note in fresh or preserved material—crescent gemmæ cups and other details as for *Marchantia*, with simple pores and other differences; mount some gemmæ in glycerine jelly and note various stages of development.

Fegatella conica (*Conocephalum conicum*); note absence of gemmæ cups, and compare other details with *Marchantia* and *Lunularia*.

II. Jungermanniales: Jungermanniaceæ

Anacrogynæ—includes *Pellia*, *Aneura*, *Mörckia* and *Blyttia* (*Pallavicinia*).

Pellia epiphylla; note in fresh or preserved material—dichotomy, young sporogonia, involucre, rhizoids, vegetative budding. Cut and mount V.S. thallus; note no air-chambers, no amphigastria, no tuberculate rhizoids, presence and form of antheridia. Cut and mount V.S. sporogonium; note and label involucre, old venter of archegonium, sporogonium with foot, seta, capsule, elaters and multicellular spores (see Fig. T 1014).

Acrogynæ—includes *Frullania* and *Cephalozia*.

Frullania sp.; note leafy material; mount material, examine dorsal and ventral surfaces; label dorsal leaves, upper lobe and lower lobe (see Fig. T 1015).

Musci

I. Sphagnales

1. **Sphagnaceæ**—*Sphagnum* spp.; examine material; mount a leaf, note chlorophyllous and hyaline cells; examine

prepared T.S. stem ; dissect reddish-brown antheridial branches ; cut L.S. young sporogonium, mount in glycerine jelly and note under L.P. and H.P. ; label foot, neck, capsule, calyptra, epidermis, rudimentary stomata, annular dehiscence line, lid, chlorenchyma, spore sac and columella (see Figs. T 1018–1022).

II. Andreaeales

2. Andreaeaceæ—*Andreaea* sp. ; examine material with capsules (see Fig. T 1023).

III. Bryales

3. Tetraphidaceæ—*Tetraphis* (see Fig. T 1024).

4. Polytrichaceæ—*Polytrichum commune* ; note and draw plant, also young sporogonium ; label seta, capsule, apophysis, calyptra, operculum, epiphragm, peristome pores. Cut and mount T.S. leafy stem ; label tissues of stem and leaf ; note no lamellæ on basal part of leaf. Examine hand sections or prepared slides of L.S. antheridial rosette, labelling perigonal leaves, paraphyses and antheridia ; also median L.S. sporogonium labelling seta, apophysis with epidermis, stomata and chlorenchyma ; capsule with wall, outer ærenchyma, trabeculæ, two-layered wall of spore sac, spore sac (cp. T.S.), inner ærenchyma, trabeculæ, columella, operculum, epiphragm, peristome (see Figs. T 1025–1027).

Eu-bryales

14. Funariaceæ—*Funaria hygrometrica* ; examine material in detail ; label in your drawings leaf, midrib, wings ; cut and mount T.S. stem ; note in material or prepared slides the details of protonema, also antheridial rosette and archegonial branch. Examine sporogonium with seta, foot, capsule and calyptra ; remove operculum and note peristome teeth. Cut L.S. capsule and mount in glycerine jelly ; label in your drawings seta, apophysis with chlorenchyma, stomata, wall and all details as Figs. T 1033–1035, see also Figs. T 1028–1032.
18. Bryaceæ—*Bryum* ; note material with capsules.
Mnium ; examine as for *Funaria*.

Note. Mosses are easily collected, dried and kept in envelopes or displayed in flat, glass-topped boxes.¹ A named collection of local mosses should be available for students, particularly in connection with ecological studies.

¹ A convenient size is 3 inches × 4 inches × $\frac{1}{4}$ inch.

CHAPTER XXXVIII

PTERIDOPHYTA

First Year Types

538. *Aspidium* (*Dryopteris filix-mas*). Stem. Examine and draw whole plant (sporophyte) of *Aspidium* (Fig. T 1058). Note stem with numerous leaf bases and adventitious roots. Cut stem transversely in half and note cut ends of vascular strands (Fig. T 1059–1061).

Leaf. Examine leaf of *Aspidium*. Note (1) rachis with ramenta ; (2) pinnae, on the under side of which are the sori. Each sorus contains a group of sporangia and the whole is covered by an indusium. Remove a few sporangia and examine under low-power (Figs. T 1069–1070). Note (1) stalk of sporangium ; (2) annulus of thickened cells and the thin part of sporangium wall (stomium) where dehiscence takes place ; (3) spores.

Gametophyte. Examine the prothallus of a fern with a hand-lens. Note and draw (1) heart-shaped thallus with the growing point between the lobes ; (2) rhizoids at the posterior end. Mount prothallus with lower surface uppermost ; examine and draw. Look for (a) antheridia showing as hemispherical outgrowths among the rhizoids ; (b) archegonia nearer the growing point (Fig. T 1075). From the fertilised egg-cell of an archegonium a new fern-plant arises. The young plant (sporophyte) grows attached to the gametophyte for a time. Note, as in Bryophyta, the very distinct alternation of generations.

539. *Pteris*. Cut T.S. rhizome of *Pteris*. Note separate vascular strands ; each strand showing (1) endodermis, (2) ring of phloem, (3) xylem with protoxylem groups (Fig. T 1062).

Examine prepared slide V.S. fertile pinna of *Pteris aquilina* (Bracken). Note different stages in formation of spores. Here the sori are marginal. On germination a spore develops into a prothallus which is the gametophyte.

Second Year Types

N.B. (1) For classification and details see Textbook, pp. 537-538; also "Origin of Land Flora," by F. O. Bower; "Extinct Plants and Problems of Evolution," by D. H. Scott.

Eligulatae

Lycopodiales

Lycopodiaceae

Lycopodium selago; note shoot erect, dichotomous, roots at base of stem, no definite strobili (sub-genus *Urostachya*). Examine T.S. stem; T.S. root; sporophyll and sporangium; L.S. fertile zone.

Lycopodium clavatum; note shoot creeping, definite strobili, spores with reticulate ridges on wall (sub-genus *Rhopalostachya*). Examine plant; note hair-like leaf-tip; T.S. stem; prepared section of branching region; L.S. stem, noting annular and spiral elements of protoxylem, also sieve-tubes with lateral sieve-plates; prepared section T.S. leaf; T.S. root; external features of strobilus; sporophyll, sporangium and spores in detail (see Figs. T 1038-1042).

Ligulatae

Selaginellaceae. *Selaginella martensii*; examine plant; note leaves in alternate pairs, one of each pair large and one small, also midrib, ligule and single chloroplast in each cell; note rhizophores, monarch in T.S.; also external features of strobili with reddish-brown microsporangia and green megasporangia. Cut, stain and mount permanently T.S. stem, T.S. rhizophore, L.S. strobilus (see Figs. T 1043-1045).

Isoetaceae. *Isoetes lacustris*; examine plant, note longitudinal grooves of stem where branching adventitious roots arise; examine prepared T.S. leaf; also L.S. base of sporophyll, noting ligule, velum or flap, sporangia with trabeculae, and heterospory (see Figs. T 1046-1047).

Lepidodendreae (fossil)

Lepidodendron; Devonian, Carboniferous, Permian. Examine cast of stem; note leaf-bases with cushion, leaf-scar with vein scar and two parichnos, also ligule scar. Examine T.S. *L. pettycurensis*; note protostele with secondary xylem. Examine L.S. *Lepidocarpon lomaxii* and cast of *Stigmaria*. See Scott's "Extinct Plants" or Seward's "Evolution of Plants" for *Lepidocarpon lomaxii* and *Miadesmia*.

Equisetales

Equisetaceae

Equisetum arvense; make drawings of prepared specimen to show rhizome with adventitious roots, fertile axis with terminal strobili, vegetative axis with branches and leaf-sheaths.

Equisetum maximum ; note hollow internodes, solid nodes, alternating leaves, longitudinal striæ on outside of stem. Cut, stain and mount permanently T.S. aerial internode.

Other species. Make diagrams of T.S. internodes of *E. palustre*, *E. hiemale*, *E. limosum* ; showing especially chlorenchyma, sclerenchyma and endodermis. Draw one vascular strand in detail. Examine prepared T.S. root *E. hiemale* ; note double endodermis and tetrarch xylem. Using any available species, examine and draw the strobilus, sporangiophore, sporangia, and spores. Note also thickenings on wall of sporangia, in fresh or prepared sections (see Figs. T 1048-1055).

Calamites (fossil) ; examine and draw a pith cast, noting grooves corresponding to vascular ridges ; also T.S. stem showing partly hollow pith, ring of vascular strands each with a carinal canal (note spiral thickenings), zone of secondary xylem and remains of phloem. Note L.S. stem showing nodal diaphragm.

Ophioglossales

(Megaphyllous, cp. microphyllous Lycopodiales and Equisetales).

Ophioglossaceæ—*Ophioglossum vulgatum* ; note erect rhizome with leaf-scars ; thick unbranched roots, usually one to each leaf-scar ; apical bud. Examine leaf with petiole, lamina, and sporangiophore (spike) with two marginal rows of sporangia, (see Fig. T 1056).

Botrychium lunaria ; note pinnatifid lamina and pinnate sporangiophore with large, nearly spherical, sporangia.

Filicales

I. **Simplices** (sporangia in each sorus arise simultaneously).

Marattiaceæ. *Angiopteris* ; note and draw details of fertile pinnae (Fig. T 1073).

Marattia ; note and draw details of pinna ; examine prepared slide of synangium, noting annulus and line of dehiscence (Fig. T 1073).

Osmundaceæ. *Osmunda regalis* (Royal Fern) ; examine fertile frond (Fig. T 1072), noting sporangia all round the reduced fertile pinnae, also absence of indusium. Dissect off sporangia ; mount in glycerine jelly and examine, noting stalk and obliquely apical characteristic annulus. Cut, stain and mount permanently T.S. petiole. Examine this section and note endodermis, phloem around xylem, adaxial protoxylem. Towards the base of the petiole the conducting strand becomes U-shaped, the protoxylem groups fuse to one and the endodermis opens to join up with the cauline endodermis

as the leaf-trace takes its place in the ring of cauline strands.

Schizaeaceæ. *Lygodium*; a tropical climber with small rhizome and long pinnate leaves. Draw a fertile pinnule to show sporangia, indusium, annulus and stonium. Make a diagram of T.S. (prepared section) of rhizome with protosteles, exarch protoxylem groups with scalariform thickening.

Aneimia; examine prepared specimen, noting two basal fertile pinnæ and sterile upper pinnæ.

Marsileaceæ. *Marsilea*; examine specimens, also prepared section of sporocarp (Figs. T 1081 and 1085). Each loculus is a sorus; note microspores and megaspores. Make a diagram of T.S. rhizome (prepared section) (see Figs. T 1083-1084).

Pilularia; examine available material as above (Figs. T 1082 and 1086).

II. Gradatæ (sporangia of each sorus arise in succession).

Salviniaceæ. *Salvinia*; examine available material, also prepared sections of sori (Figs. T 1087-1089).

Azolla; examine available material; cut T.S. plant and note endophytic *Anabaena* filaments.

Hymenophyllaceæ. *Trichomanes radicans* (Killarney Filmy Fern); examine elongated placenta, indusium. Cut L.S. sorus and note sporangia, basipetal succession, annulus and stonium.

III. Mixtæ (sporangia of each sorus arise sporadically).

1. Davallioidææ. *Davallia*; sorus marginal, indusium cup-shaped.

2. Pteroidææ. *Pteris*; sori coalescent on margin. Draw T.S. pinna of *P. aquilina*, noting details of sporangium, annulus and stonium (Figs. T 1068-1070); also rhizome T.S. under L.P. (Fig. T 1062), one meristele under H.P.; and T.S. root from prepared slide (Fig. T 1066).

3. Gymnogrammoideææ. *Adiantum*; sorus marginal with reflexed lobe of lamina at or near apex of pinna or pinnule.

4. Dryopteroidææ. *Dryopteris (Aspidium) filix-mas*; sorus orbicular; indusium peltate or reniform (Fig. T 1069). Examine and mount prothallus, note archegonia, antheridia, rhizoids, etc. (cp. Figs. T 1074-1080). Examine rhizome, draw external features, also T.S.; dissect out meristemes; note prepared specimen of dictyostele; draw one meristeme in T.S. and L.S. under H.P. (Figs. T 1059-1067). *Dryopteris phegopteris (Polypodium in part)*; sorus orbicular; indusium absent.

5. Asplenoidea. *Asplenium* ; sorus elongated, not marginal ; indusium lateral.
6. Blechnoidea. *Blechnum spicant* ; sorus coalescent along midrib of pinna, not marginal.

Pteridospermæ

All fossil, maximum in Coal Measures, see Textbook, p. 531, also Scott's " Extinct Plants."

Lyginodendron oldhamium. Examine T.S. stem ; note monostele, large pith, groups of primary xylem, mesarch protoxylem (cp. *Cycas* below), broad zone of secondary xylem with medullary rays, cambium, zone of secondary phloem, primary phloem if present, pericycle with leaf-traces, thin-walled inner cortex, outer cortex with interlacing strands of sclerenchyma (dark radial bands in T.S.) ; also periderm if present. This type of cortex is characteristic of the group. Note five leaf-traces with two-fifths phyllotaxis ; leaf-trace collateral in cortex becomes concentric in petiole (cp. *Osmunda*).

Seed ; examine prepared sections of seed of *L. oldhamium* (*Lagenostoma lomaxii*) ; note atropous ovule of Cycadean type, one integument adhering to nucellus except at apex, pollen chamber in apex of nucellus (cp. *Ginkgo*, *Cycas*, etc.).

CHAPTER XXXIX

GYMNOSPERMÆ

First Year Type

Pinus.

540. Examine material of *Pinus* with male inflorescences ; also old female cones showing the two naked seeds which are not enclosed in an ovary as in Angiosperms. Examine and draw prepared slide L.S. ovule of *Pinus*. Note external features of short shoots (Fig. T 1094).

541. (a) Cut T.S. leaf (see Figs. T 1095–1097) and note all details in section.

(b) Cut L.S. leaf and note plates of chlorenchyma with large air spaces between the plates.

542. Cut T.S. and L.S., both radial and tangential, of stem ; note anatomical and histological details (Figs. T 1090–1098).

Second Year Types

N.B. (1) For classification see below, also “ Flowering Plants and Ferns,” by J. C. Willis (Camb. Univ. Press). For details and identification of conifers see “ Handbook of Coniferæ,” by Dallimore and Jackson ; or Bean’s “ Shrubs and Trees Hardy in the British Isles.”

(2) Drawings should be made of all observed general forms and structural details.

Cycadales

Tropical and subtropical, see Textbook, p. 554.

Cycas revoluta ; note general habit of plant and of leaf. Examine cut end of petiole, note curved group of conducting strands. Cut, stain and mount permanently T.S. of petiole ; make a diagram of the entire section ; note under H.P. one bundle in detail with mesarch protoxylem and slightly developed centrifugal primary xylem. Cut, stain and mount permanently T.S. pinna ; note and draw part in detail Examine megasporophylls (other genera have these in cones) ; note reduced pinnæ. Examine prepared slide L.S. ovule (*cp. Lyginodendron* seed). Note and draw details of microsporophyll from compact cones (see Fig. T 1124).

Ginkgoales

Monotypic, except for fossils ; only known cultivated, originally in China and Japan.

Ginkgo biloba (Maidenhair Tree); examine plant ; note deciduous leaves on short shoots. Note leaves with lobes and dichotomous venation ; also prepared specimens of microsporophylls and carpellate twigs (Fig. T 1125).

Gnetales

Welwitschia mirabilis ; examine prepared specimen ; also prepared slide showing floral structure (Fig. T 1126).

Ephedra sinica ; recently of importance as a medicinal plant yielding ephedrine. Examine prepared specimen of shoot ; note pairs of scale-leaves and switch-habit ; also prepared sections of young stems.

Coniferales

Taxaceæ (mostly diœcious)

Podocarpoideæ—includes *Podocarpus* (see Textbook, p. 162).

Taxoideæ. *Taxus baccata* ; diœcious ; examine twig and note leaf-bases and short twisted petioles (“ P.-I. P. L.” 182). Examine staminate cones in axils of last year’s leaves ; dissect and examine stamen and pollen-grains (Figs. T 1119–1120). Note also sub-terminal female flowers and prepared L.S. (Fig. T 1121). Cut L.S. arillate fruit and draw details (Figs. T 1122–1121).

Pinaceæ (mostly monœcious)

I. Araucariæ—includes *Araucaria* (Monkey Puzzle).

II. Abietæ—includes *Picea* (spruce) with four-angled leaves, persistent projecting leaf-bases, and pendulous cones ; *Tsuga* (Hemlock Spruce) similar, but with flat leaves (Fig. T 1112) ; *Pseudotsuga* (*P. douglasii* = Douglas Fir) with flat leaves, each with two lateral resin-canals, also large bracts with three-lobed ends in ripe cones ; *Abies* similar, but with cone erect, bracts usually short and scales deciduous ; *Larix* with short shoots, deciduous foliage leaves, cones with persistent scales and long bracts ; also

Pinus sylvestris. Examine this in detail (see Figs. T 1090–1106). Note long and short shoots, pairs of foliage leaves, male and female cones in external detail ; prepared T.S. young stem. Cut, stain and mount permanently T.S. and radial L.S. of old stem ; also tangential L.S. in the region of the wood. Note in T.S. annual rings, pits, and rays ; draw secondary wood under H.P. Note in R.L.S. tracheids, pits, sieve tubes with lateral sieve-plates, and details of medullary rays ; in T.L.S. tracheids and pits. Cut, stain and mount permanently T.S. and L.S. leaf ; draw line diagrams under L.P. and details under H.P. ; note

plates of chlorenchyma in L.S. Examine prepared T.S. root, note resin-canals, protoxylem groups, etc., as in Fig. T 1098.

Cones; examine and dissect male cone; draw small cone, sporophyll or stamen, and pollen-grains; examine prepared series of female cones in first, second and third year stages. Pollination occurs in June of first year, fertilisation in June or July of second year. Examine and dissect the cones supplied; note scales, ovules and bracts. Cut L.S. ovule from second-year cone; mount and examine in detail (Figs. T 1101-1102B).

III. Taxodiæ—includes *Sciadopitys* (Umbrella Pine) with needles fused along margin; *Sequoia* with shield-shaped or double carpellary scales.

IV. Cupresseæ—includes *Thuja* with imbricate ovuliferous scales in a woody cone; *Cupressus* (Cypress) with scales peltate and strongly woody when ripe (Fig. T 1113); also *Juniperus* with berry-like fruits (Figs. T 1114-1116).

CHAPTER XL

ANGIOSPERMÆ

THE corresponding chapter of the Textbook has been found very suitable for use in practical classes, but details are given below of some first-year types, together with very brief notes on other types and on a few points of systematic anatomy which are not given in the Textbook.

N.B.—For details of classification see “Flowering Plants and Ferns,” by J. C. Willis; or “Classification of Flowering Plants,” by A. B. Rendle, Camb. Univ. Press. For Anatomy see Solereder’s “Systematische Anatomie -der Dicotyledonen” (Enke); “-der Monokotyledonen” (Borntraeger).

Stomatal types are—

I. With no special accessory cells (Ranunculaceæ).

II. With 2 accessory cells derived from the guard-cells (as in many Monocotyledons).

III. (a) With 4 or more accessory cells derived as in II. (*Ficus*, some Monocotyledons, Coniferales and Cycadales).

(b) With 3 or more accessory cells derived from stomatal mother-cell (Cruciferae).

IV.–V. With 2 accessory cells derived from the stomatal mother-cell; in two positions thus—

IV. Parallel to the guard-cells (Rubiaceæ).

V. Lying around the ends of the pair of guard-cells (Caryophyllaceæ).¹

DICOTYLEDONES

Archichlamydeæ

Salicaceæ. Fruit a 2-valved loculicidal capsule with hairy seeds.

Salix, willow; ♂ Po A2-5; ♀ Po G(2); nectary posterior, stigmas small, entomophilous.

Populus, poplar; ♂ P a lobed cup, A10-30; ♀ P a lobed cup G(2); no nectary, stigmas large, anemophilous.

¹ For types of Hairs see Ex. 73. For types of Calcium Oxalate Crystals see Ex. 31.

Anatomy. Glandular hairs absent; stomata type IV, on both sides of leaf in *Salix*. *Populus* shows sclerenchyma in bark, *Salix* none. Calcium oxalate in single and cluster crystals.

Myricaceæ. Fruit a nut.

Myrica gale, bog myrtle; ♂ Po A2-16, usually 4, 1 bract and no bracteoles; ♀ Po \bar{G} (2) ovary unilocular with 1 atropous ovule, 1 bract and 2 bracteoles.

Anatomy. Oleo-resin glands near base of under side of bracts. Stomata of type IIIb, sometimes with 5-10 accessory cells which may be papillose. Sclerenchyma pericyclic. Crystals as in Salicaceæ.

Betulaceæ. Fruit a nut. Inflorescence terminal (Fig. T 1139-1140). Ovary inferior, 2-celled with 1 ovule in each loculus; 1 integument. For pollen grains see Fig. 25.

Betula alba, birch; ♂ 3 frs., α β bracteoles, P2 A2; ♀ 3 frs., α β bracteoles, Po \bar{G} (2).

Corylus avellana, hazel; ♂ median fl. only, α β bracteoles, Po A4; ♀ 2 lateral frs. only, all bracteoles, Po \bar{G} (2).

Carpinus betulus, hornbeam; ♂ 2 lateral frs. only, Po A4-12, all bracteoles absent; ♀ 2 lateral frs. only, all bracteoles present and fused, P4 epigynous \bar{G} (2).

Alnus glutinosa, alder; ♂ 3 frs., α' bracteoles absent, others fused, P4 A4; ♀ 2 lateral frs. only, α' bracteoles absent, Po \bar{G} (2).

Anatomy. Hairs glandular of various types. Stomata type I. Crystals single or clustered. Vessels often with scalariform perforations; vessels in short radial groups.

Fagaceæ. Fruit a nut. Inflorescence axillary (*cp.* Fig. T 1139-1140). Ovary inferior, 3-celled, with 2 ovules in each loculus; 2 integuments.

Fagus sylvatica, beech; ♂ in cymose head, each dichasium with 3 frs., α β bracteoles absent, P5-8 A5-8; ♀ 2 lateral frs. only, α β bracteoles absent, P6 \bar{G} (3), 2 nuts in cupule.

Quercus, oak; ♂ in catkins, median fl. only, α' β' bracteoles absent, P5-9 A5-9; ♀ frs. solitary, P5-6 \bar{G} (3), 1 nut in cupule.

Castanea vulgaris, sweet chestnut; ♂ in catkins, each dichasium with 3-7 frs., all bracteoles present, P6 A10-12; ♀ dichasia solitary, each with 3 frs., all bracteoles present, P6 \bar{G} (3), 3 nuts in cupule.

Anatomy. Similar to Betulaceæ, but vessels solitary scattered (Beech) or tangentially grouped (Oak and Chestnut).

Ulmaceæ. *Ulmus campestris*, elm; frs. $\bar{\zeta}$, appearing in reddish-purple clusters before leaves, each cluster has 10-12 sterile bracts, then several fertile bracts each with 1 fl., P4-5 A4-5 anteposed \bar{G} (2). Fruit a samara.

Anatomy. Hairs simple and glandular. Stomata in *Ulmus* type I. Crystals single or clustered. Latex absent.

Moraceæ. *Morus alba*, white mulberry; fruit, note P4 G (2) in pseudocarp.

Ficus carica, fig; note in pseudocarpic syconus, ♂ flrs., P4 A1-2; also short-styled and long-styled ♀ flrs., P4 G(2), each fruit developed to a one-seeded drupel with yellow sclerocarp (see "P.-l. P. L.," No. 187).

Humulus lupulus, hop; diœcious, ♂ P5 A5; ♀ P1 G(2) in strobiles, fruit an achene, seeds endospermic.

Anatomy. Latex tubes. Cystoliths common (*Ficus*). Hairs various, simple or glandular (*Humulus*) or with cystoliths. Stomata type I, or in *Ficus* IIIa.

Urticaceæ. *Urtica dioica*, nettle; diœcious; ♂ P4, A4 with explosive dehiscence of anthers; ♀ P(4) G1. *U. urens*, monoœcious.

Anatomy. Hairs simple and stinging. Cystoliths common. Stomata often on both sides of leaf, type I in *Urtica*, III in other genera. Crystals single or clustered. Bast fibres very long.

Polygonaceæ

Rumicoideæ. *Rumex*; P3 + 3 A6 + 0 G(3) with feathery stigmas, anemophilous. *Rheum*; P3 + 3 A6 + 3 G(3), entomophilous.

Polygonoideæ. *Polygonum*; P5 petaloid A various.

Anatomy. Hairs simple, branched or glandular. Stomata type I. Crystals usually clustered, sometimes single or raphides. Resin cells and tannin cells occur in *Polygonum*; medullary bundles, concentric or with inverse orientation in *Rumex* and *Rheum*; sub-epidermal strands of sclerenchyma in *P. aviculare* and *P. equisetiforme*.

Caryophyllaceæ. K5 or (5) C5 A5 + 5 obdiplostemonous G (5-3) or 1. Nodes swollen.

Silenoideæ. K(5), corolla tube long. *Silene* G(3); *Lychnis* G(5); *Dianthus* G(4).

Alsinoideæ. K5, corolla tube short. *Stellaria* G(3-4); *Cerastium* G(5); *Spergula* G(5); *Spergularia* G(3).

Aberrant. *Sagina* 4 or 5-merous; *Paronychia* K5 Co A5 G1.

Anatomy. Clothing hairs and glandular hairs of various types are common. Stomata type V. Crystals clustered, single large crystals rare or absent. Leaves often centric. Sclerenchyma rare.

Chenopodiaceæ. Stamens anteposed; ovary unilocular with one campylotropous ovule.

Chenopodium; P5 or (5) A1-5 G(2);
Atriplex; ♂ P5 A5; ♀ Po G(2);
Beta; P(5) A5 G(5) or (2) semi-inf.;
Suaeda; P5 A5 G(2-3); leaves semi-cylindrical.

Salsola ; P(5) A5 \underline{G} (2) ; leaves cylindrical spiny.

Salicornia ; P(3) A1 -2 \underline{G} (2-3), flrs. axillary reduced ; leaves as leaf-bases surrounding stem.

Anatomy. Hairs various, simple, with papillate walls, or ballooned or branched and stellate, also candelabra ; seldom glandular. Stomata types I or IV, often with the guard-cells lying at right angles to the long axis of leaf or stem. Crystals clustered or sandy. Anomalous root and stem structures common, with concentric zones of xylem and phloem or with bundles embedded regularly or irregularly in prosenchyma. Pericycle often sclerenchymatous.

Ranunculaceæ

543. Examine flower of Buttercup (*Ranunculus*). Note following organs : (a) calyx of 5 sepals, polysepalous ; (b) corolla of 5 petals, polypetalous ; (c) andrœcium of numerous spirally arranged stamens ; (d) gynœcium composed of numerous free (apocarpous) carpels. Dissect a flower and draw on a large scale : (i.) a single petal with a nectary at base ; (ii.) a single stamen ; (iii.) a single carpel. Describe the flower ; recording the details as a floral formula, a floral diagram and a longitudinal elevation. Write a description of the method of pollination.

(1) Pæoniæ : no nectary leaves. *Pæonia* \underline{G} 2, follicles.

(2) Helleboreæ : follicles. *Caltha* P5, \underline{G} 5, with nectary in lateral wall near base of each carpel. *Trollius* P5, *Helleborus* P5 and *Eranthis* P6 with involucre, also tubular nectaries between stamens and perianth. *Nigella*, involucre of 5 bracteoles \underline{G} (5). *Aquilegia* regular with five-spur nectaries. *Delphinium* K5 one-spurred, C5 two-spurred. *Aconitum* K5 petaloid zygomorphic, C5 two narrow nectaries, three hair-like or absent.

(3) Anemoneæ : achenes. *Anemone* involucre, P6-8. *Clematis* P4 or 8 petaloid, nectary absent. *Ranunculus* K5 C5 A ∞ hypogynous G ∞ ; *R. ficaria* K3 C7-9. *Thalictrum* unisexual P4-5 sepaloid caducous.

Anatomy. Hairs simple, rarely glandular ; also hydathodes. Stomata type I. Calcium oxalate not frequent. H-shaped palisade cells common in leaf. Pericyclic sclerenchyma common ; xylem often V- or U-shaped in T.S., partly enclosing phloem ; vessel perforations simple, scalariform in *Pæonia*. Supernumerary bundles in stem of *Thalictrum*, *Actæa* and *Cimicifuga* ; medullary strands in *Anemone japonica*, and wide medullary rays in *Clematis*.

Cruciferæ

544. Examine and dissect a flower of *Cheiranthus* (Wallflower). Is the flower hypogynous, perigynous or epigynous? Note (a) the inflorescence, a raceme; (b) calyx of 4 sepals, the lateral sepals saccate; (c) corolla of 4 petals placed diagonally; (d) andrœcium of 6 stamens (4 long and 2 short = tetradynamous); (note the green nectaries opposite the bases of the 2 saccate sepals); (e) syncarpous gynœcium of 2 carpels consisting of the elongated ovary, short style and two stigmas. The ovary is bilocular with parietal placentation. Describe the flower; record the longitudinal elevation, floral formula and floral diagram.

Sinapeæ. Hairs simple or absent; no glandular hairs. *Lepidium*, *Iberis*, *Cochlearia*, *Alliaria*, *Sisymbrium*, *Cakile*, *Sinapis*, *Brassica*, *Raphanus*, *Crambe*, *Nasturtium*, *Cardamine*.

Hesperideæ. Hairs usually branched; glandular hairs frequent. *Capsella*, *Draba*, *Arabis*, *Erysimum*, *Cheiranthus*, *Alyssum*, *Hesperis*, *Matthiola*.

Anatomy. Hairs simple, two-armed, stellate, candelabra or peltate, with or without calcium carbonate incrustations, glandular in some Hesperideæ. Stomata type IIIb mainly (Fig. T 264). Medullary rays usually lignified. Myrosin cells are usual.

Papaveraceæ

Papaveroideæ. Latex; K2 caducous, C4 not spurred. A ∞ G(2- ∞). *Papaver* G(∞) with rayed sessile stigma and porous capsule. *Meconopsis cambrica* G(5) with twisted style. *Chelidonium majus* (greater celandine); *Glaucium* (horned poppy); *Eschscholtzia* G(2), fruit a siliquæform capsule (Fig. T 1191); *Bocconia*, shrub K2 Co A ∞ G(2).

Hypecoideæ. See Textbook.

Fumarioideæ. Latex absent; K2, C4 spurred, A2 each branched with one whole and two half anthers, G(2); *Dicentra* and *Corydalis* with fruit a capsule; *Fumaria* with fruit a nut.

Anatomy. Hairs simple or biseriate or multiseriate emergences, also branched, sometimes very large. Stomata type I. Crystals absent except in *Bocconia*. Latex in vessels (*Papaver*, *Chelidonium*), or sacs (*Eschscholtzia*, *Glaucium*) or solitary cells.

Rosaceæ

545. A. *Potentilla anserina* (Silverweed). Note and draw stipulate compound leaves. Examine the flower and

observe : (a) calyx of 5 sepals with an epicalyx ; (b) corolla of 5 petals ; (c) numerous perigynous stamens ; (d) numerous separate carpels. Record the floral formula, floral diagram and longitudinal elevation.

B. *Cratægus* (Hawthorn). Note and draw leaves with stipules. Examine and draw flower ; compare with *Potentilla*. Note apparently epigynous stamens which are really perigynous.

Spiræoideæ—carpels usually 5 in a whorl, receptacle flat, fruit an etærio of follicles, stamens with a broad base. *Spiræa*.

Pomoideæ—carpels 5-2 syncarpous, fruit a pomc. *Pyrus* and *Cratægus*.

Rosoideæ—carpels many, usually spirally arranged, sometimes on a carpophore. *Rhodotypos* A ∞ , G2 as in saxifrage ; *Kerria japonica* ; *Rubus* fruit an etærio of drupels ; *Fragaria*, *Potentilla*, *Geum*, *Dryas*, fruit of achenes, epicalyx usually present ; *Ulmaria* fruit 3-12 follicles, stamens with a narrow base ; *Alchemilla* K4 C0 A4 G1, style *gynobasic* ; *Agrimonia* persistent receptacle with numerous hooks ; *Poterium* unisexual, anemophilous ; also *Rosa*, with persistent fleshy receptacle.

Prunoideæ—carpel solitary, fruit a drupe. *Prunus*.

Anatomy. Hairs simple uniseriate. Stomata type I. (or IV. in Chrysobalanoideæ). Calcium oxalate crystals usually single prisms. Scalariform perforations occur in the vessels of the woody forms which also have isolated groups or a continuous ring of fibres in the pericycle ; primary cortex usually collenchymatous.

Leguminosæ

546. Examine the flower of *Laburnum*. Observe the zygomorphic form and note the following parts : (a) calyx of 5 gamosepalous sepals forming two lips ; (b) corolla polypetalous, of 5 petals, 1 large standard (posterior), 2 lateral wings and 2 anterior petals cohering below to form the keel, which encloses the essential organs ; (c) andræcium of 10 stamens united by their filaments to form a tube enclosing (d) the gynæcium composed of one carpel. Open the carpel ; note parietal placentation and numerous ovules. Make separate drawings of each of the above parts dissected out ; record the floral formula, floral diagram and longitudinal elevation.

I. Papilionatæ—K5 ascendingly imbricate, odd sepal anterior, C5 descendingly imbricate, A (10) or (9) + 1 \bar{G} 1. *Myroxyton* all stamens free; fruit a lomentum in *Onobrychis*, *Arachis*, etc.; fruit an indehiscent legume in *Pterocarpus*; fruit a dehiscent legume in Genistæ (shrubs with 3 entire leaflets), *Lupinus*, *Genista*, *Laburnum*, *Ulex*, *Cytisus*; in Galegeæ, e.g. *Indigofera*, *Robinia*, *Colutea*, *Astragalus*; in Lotææ (with 5 entire leaflets), *Lotus*, *Anthyllis*; in Trifoliceæ (with 3 toothed leaflets), *Ononis* A (10), and *Medicago*, *Melilotus*, *Trifolium* A (9) + 1; in Viciae (leaves pinnate, ending in tendril or bristle), *Vicia*, *Lathyrus*, *Pisum*, *Lens*, *Abrus*; in Phascolææ (leaves pinnate, usually with terminal leaflets), *Phaseolus*, *Glycine*, *Butea*, *Mucuna*, etc.

II. Cæsalpinoideæ—K5 or (5) C5, both ascendingly imbricate, A 10 \bar{G} 1 mostly exotic; see Textbook and "Willis." *Cassia* asymmetric with nearly equal petals and dimorphic stamens.

III. Mimosoideæ—K (4) C4, valvate small, A4-∞ \bar{G} 1. *Acacia* A ∞, free; *Mimosa*, A4-8.

Anatomy. All have simple perforations in vessels and simple pits in the prosenchyma. Hairs are simple or multicellular, sometimes branched in group I.; glandular hairs of various types occur in groups II. and III. Stomata types I. and IV. occur in groups I. and II.; type IV. is common in groups II. and III. Calcium oxalate crystals are usually single, rhomboidal or styloid; clustered crystals are rare, but occur in *Mimosa*. Anomalous secondary thickening is found in *Bauhinia* and other lianes.

Saxifragaceæ. Flrs. regular, perigynous; K5, C5, A5 + 5 obdip., G2 semi-inferior, partly syncarpous. Several variations from the above occur.

Saxifraga sarmentosa corolla zygomorphic; *Tolmiea menziesii* anterior split in deep receptacular tube and only 3 posterior stamens present; *Chrysosplenium* K4 Co A8 \bar{G} (2); *Parnassia* A5 with 5 branched staminodes; *Heuchera* C absent usually; *Deutzia* \bar{G} (3-5); *Hydrangea* K4 C4 A8 \bar{G} (2-5); *Philadelphus* A20-40 \bar{G} (3-5); *Escallonia* A5 \bar{G} (2-3); *Ribes* \bar{G} (2); *Bauera* A4-∞.

Anatomy. Hairs simple, unicellular or tufted; stellate in *Deutzia*; chalk glands common, also glandular hairs of various types. Stomata type I or in some *Hydrangea* spp., type IV. Crystals occur as raphides (*Hydrangea*), styloids (*Escallonia*), also as simple or clustered crystals. Scalariform and simple perforations are found in the xylem; medullary rays wide in woody forms.

Geraniaceæ. K5, C5, A5–10–15 filaments fused at base, obdip., $\bar{G}(5)$ or less; styles united; ovules anatropous, raphe adaxial, micropyle upwards (Fig. T 1127). Fruit a beaked regma. Leaves stipulate.

Geranium A5 + 5 with 5 nectaries at base; *Erodium* A5 with 5 staminodes, corolla sometimes zygomorphic.

Pelargonium A5 trifold and petaloid at base of filament, or some of the 10 staminodes may be fertile; receptacle with hollow spur and nectary near base of long tube.

Anatomy. Hairs simple or glandular, sometimes very long. Stomata type I. Calcium oxalate as sphaeroidal clusters or single. Vessels with simple perforations; medullary bundles in *Geranium*; peculiar thickenings occur on the radial walls of the exodermis.

Oxalidaceæ. K5, C5 or (5), A5 + 5 obdip., $\bar{G}(5)$ styles free. Fruit a capsule or berry. Leaves exstipulate.

Oxalis—fruit an explosively loculicidal capsule, seed with aril.

Anatomy. As for Geraniaceæ.

Tropæolaceæ. K5, C5 perigynous A4 + 4 upper short and lower long sets of 4 maturing successively, $\bar{G}(3)$. Fruit an unbeaked regma. Leaves usually peltate with tendril petioles.

Tropæolum, flr. strongly zygomorphic, with posterior spur formed by receptacle.

Anatomy. Like Geraniaceæ, with myrosin cells present.

Rutaceæ. K5–4, C5–4, A10–8 rarely 2– ∞ $\bar{G}(5-4)$ rarely (1– ∞), ovary typically 5-celled with large disc below. Leaves with glandular dots, often aromatic.

Ruta, 5- or 4-merous; *Correa*, corolla sympetalous, flr. 4-merous; *Boronia*, *Eriostemon*, *Choisya*, *Skimmia*, flr. 5-merous; *Citrus* and *Ægle* $\bar{G}(10)$ or more, fruit a berry with internal emergences containing abundant juice.

Anatomy. Hairs, branched or stellate, also glandular. Stomata type I or occasionally type IV. Crystals of all forms occur. Schizogenous secretory glands occur in the leaf and stem, usually as characteristic oil glands. Phellogen usually arises near the outside of the cortex.

Euphorbiaceæ. Flrs. various, $\bar{G}(3)$ rarely (2); ovules of *Geranium* type; fruit, a regma.

Euphorbia; cyathium; ♂ Po A1 in cincinni, tissues of filament and flower-stalk slightly different; ♀ Po $\bar{G}(3)$; bracts may appear as "perianth" to flower-like inflorescence.

Mercurialis; dioecious; ♂ P8 A9–12, ♀ P8 $\bar{G}(2)$.

Dalechampia; infr. complex; ♂ P8 A9–14, ♀ P6 $\bar{G}(3)$.

Acalypha; long monœcious spike.

Anatomy. Hairs simple, branched or glandular. Stomata

usually types IIIb or IV. Crystals single or clustered. Phellogen often sub-epidermal; latex tubes usually unbranched, common; branched tubes occur in *Hevea* and *Manihot*; various other secretory ducts may be found.

Buxaceæ. *Buxus sempervirens*; flrs. in heads with terminal ♀ K4-5 Co G(3) and lateral ♂ K4 Co A4, ovule with abaxial raphe and micropyle upwards (Fig. T 1129). Fruit, a loculicidal explosive capsule. Stem hairy, partially covered with glabrous leaf-bases ("P-l. P. L.," No. 181).

Anatomy. Hairs simple. Stomata type I. Crystals usually clustered, absent from leaf. In *Buxus* phellogen is sub-epidermal; secretory cavities and oil glands absent.

Limnanthaceæ. *Limnanthes douglasii*; K5, C5, A5 + 5 G(5), ovule with adaxial raphe and micropyle downwards (Fig. T 1128). Fruit, a schizocarp.

Anatomy. Like Geraniaceæ, with myrosin cells present.

Balsaminaceæ. *Impatiens*; K5 posterior sepal spurred, petaloid; two anterior sepals reduced or absent, C5 lateral petals fused in pairs. A(5) filaments united and anthers adhering to form a cap over stigma, G(5); ovules of *Buxus* type. Fruit, an explosive capsule.

Anatomy. Hairs simple or glandular. Stomata type I. Calcium oxalate in raphides. Isolated vessels with annular or spiral thickening occur in the pith.

Malvaceæ. K5 or (5) often with epicalyx, C5, A(∞) with united filaments, G1-(∞). Fruit, a carcerule or capsule.

Malva; K(5) with epicalyx of 3 leaves, C5 A(∞) G(∞); carcerulus.

Lavatera arborea; as above, with 3 epicalyx leaves connate.

Althæa; as above with 6-9 epicalyx segments connate (see "P-l. P. L.," Nos. 62 and 132).

Anatomy. Hairs stellate or glandular. Stomata type I. Crystals simple or clustered. Mucilage cells in epidermis and in ground tissue of axis and leaf; phloem characteristically wedge-shaped; cortex three-layered.

Violaceæ. *Viola*; K5 C5 anterior petal often spurred, A5 with membranous apical appendages, two anterior stamens often with green nectary spurs, G(3) ovary unilocular, placentation parietal; fruit a loculicidal capsule.

Anatomy. Hairs simple, tufted and glandular. Stomata types IIIb or IV, sometimes both. Crystals simple or clustered. Epidermal mucilage cells occur.

Myrtaceæ. K4-5 C4-5 A∞ usually bent inwards in bud, G(∞-2) or 1, inferior or semi-inferior.

Myrtoideæ. *Eugenia caryophyllata*; examine cloves which have been softened with hot water; K4 persistent C4 A ∞ G(2).

Pimenta; examine all-spice berries which have been softened in hot water (see Ex. 599).

Leptospermoideæ. *Eucalyptus*; K4 inconspicuous teeth or 0, C(4) caducous as a thick cap, A ∞ G4-6. Fruit a capsule, dehiscing by slits in upper surface; seeds elongated. Also *Leptospermum*.

Anatomy. Hairs simple, seldom glandular. Stomata types I or IV. Crystals clustered or single. Tannin cells and oil-glands or secretory cavities in mesophyll, giving bright dots in leaf; perimedullary ploem in stem.

Umbelliferae. K5 C5 A5 Ḡ (2); sepals or petals rarely absent.

547. From compound umbel of *Anthriscus* or *Heracleum* remove a single flower. Find out whether it is hypogynous, perigynous or epigynous. Note (a) calyx of 5 sepals; (b) corolla of 5 petals, the peripheral one sometimes largest; (c) andrœcium of 5 free stamens; (d) gynœcium composed of 2 carpels in the median plane, 2 stigmas arising from a glandular nectary, ovary inferior bilocular. The protandrous condition renders self-fertilisation impossible. Record the floral formula, floral diagram and longitudinal elevation. For classification see below and Textbook.

Heterosciadicæ—umbel simple; vittæ absent or indistinct.

Hydrocotyle leaf peltate; *Eryngium* leaf spiny; *Astrantia* flrs. ♀ and ♂, cremocarp with white teeth ("P-l. P. L.," No. 111); *Sanicula* flrs. in cymose head.

Haplozygiæ—umbel compound, cremocarp with no secondary ridges; vittæ usual.

With commissure narrow—*Chærophyllum* (fruit not beaked); *Anthriscus* (fruit beaked); *Smyrniun* (flrs. yellowish); *Conium* (flrs. white, vittæ absent, endosperm grooved); *Bupleurum* (leaves simple, linear or ovate):

With semicircular mericarps in T.S.—*Apium*, *Petroselinum*, *Carum*, *Crithmum*, *Pimpinella*, *Fœniculum*, *Æthusa*, *Cicuta*, *Cenanthe*:

With flattened, winged mericarps—*Angelica*, *Ferula*, *Peucedanum*, *Pastinaca*.

Diplozygiæ—umbel compound, cremocarp with secondary ridges, vittæ usual.

Coriandrum (primary ridges wavy, secondary ridges

straight and more conspicuous); *Daucus* (prominent primary ridges with spines, less conspicuous secondary ridges); *Cuminum* (primary ridges, five, prominent; secondary ridges, four, rather wider, flattish and very hairy).

Anatomy. Hairs simple, sometimes very large, rarely branched; glandular hairs absent. Stomata type I usually. Crystals usually clustered; sometimes single in the axis; microspheroidal crystals occur in many of the fruits. Schizogenous gum-resin canals; outer cortex often collenchymatous; petiole often with scattered vascular strands. Various abnormalities occur in the roots and rhizomes.

Sympetalæ

Ericaceæ. K4-5 C (4-5) A8-10 obdip., pollen in tetrads, G (4-5) superior or inferior, 4-5 celled.

I. Rhododendroideæ. Seeds often winged. Corolla not persistent.

Rhododendron zygomorphic with exserted stamens; *Azalea* leaves deciduous; *Kalmia* regular, A5 with explosive dehiscence of anthers; *Dabæcia* C toothed, A8; *Ledum* petals free.

II. Arbutoideæ. Seeds not winged; corolla not persistent. Ovary superior.

Andromeda with cup-shaped corolla and small calyx; also *Gaultheria* with fleshy calyx around capsule, and horned anthers; *Pernettya* with dry calyx at base of persistent berry; with long horned anthers and fruit a berry, *Arbutus* and *Arctostaphylos*.

III. Vaccinioideæ. As II, but ovary inferior. *Vaccinium* corolla urceolate G(5); and *Oxycoccus* corolla rotate G(4) (see "P-l. P. L.," Nos. 159-160); anthers spurred or horned.

IV. Ericoideæ. Seeds not winged; corolla persistent.

Erica K4 small green C(4) A4 + 4 horned G(4), capsule loculicidal; *Calluna* K4 petaloid larger than petals with 4 small green bracts outside, C(4) nearly free, A4 + 4, G(4), capsule septicial.

For vegetative differences see "P-l. P. L.," Nos. 85-86.

Anatomy. Hairs simple, branched or two- to many-seriate, also glandular. Stomata usually type I, but type IV in Vaccinioideæ. Crystals clustered or single. Vessel-perforations scalariform, reticulate, or simple; lumen small; walls with small bordered pits. Cuticle often thick on leaves.

Primulaceæ. K (5) C (5) A5 G (5); fruit a capsule.

Primula; examined as a type (see Ex. 335); *Lysimachia* C almost polypetalous; *Glaurx* C absent; *Cyclamen* C recurved;

Hottonia C fimbriate; *Coris* C zygomorphic K spiny; *Trientalis* K(7) C(7) A7 G(7) leaves in one whorl; *Anagallis* (5-merous), and *Centunculus* (4-merous) have the fruit a pyxidium; *Samolus* G semi-inferior.

Anatomy. Hairs simple, branched or glandular, with long stalk or short stalk and 1- to 4-celled head. Stomata type I, often on both sides of leaf. Calcium oxalate crystals rare. Secretory cells sometimes red, sometimes with sphaero-crystalline contents. Endodermal cells with characteristic Casparian dots on radial walls.

Gentianaceæ. K (5) C (5) A5 G (2).

I. Gentianoideæ. Perimedullary phloem, imbricate corolla.

Gentiana, *Erythraea* typical; *Chlora* (*Blackstonia*) 8-merous, with G(2-4).

II. Menyanthoideæ. No perimedullary phloem; induplicate valvate corolla.

Menyanthes with median fringe on each petal; *Villarsia* (*Limnanthemum*) with basal fringes on each petal.

Anatomy. Hairs usually simple; intercellular in II. Stomata type I. Calcium oxalate crystals and secretory cells absent. Perimedullary phloem present in I. Vessel-perforations usually simple; lignified groups of bast-fibres are common.

Boraginaceæ. Inflorescence a boragoid; K (5) C (5) A5 G (2) fr. usually regular; fruit a carcerulus with 4 nutlets, rarely a drupe as in *Heliotropium*.

IV. Boraginoideæ. Style gynobasic; carcerulus. *Cynoglossum* and *Echinosperrum* have hooked fruits; epipetalous staminodal ligules are variously developed in *Symphytum*, *Borago*, *Anchusa*, *Alkanna*, *Pulmonaria*, *Myosotis* and *Lithospermum*. *Echium* is gynodioecious with zygomorphic corolla.

Anatomy. Hairs simple, unicellular, cystolithic, often with solid layered points, rough walls and bulbous bases; also short or long glandular types usually with unicellular heads. Stomata type I or IIIb. Crystals single, clustered or sandy. Vascular bundles bicollateral with perimedullary phloem.

Labiataæ. Inflorescence a verticillaster. K (5) reg. or two-lipped, C (5) usually two-lipped, A4 didynamous or nearly equal, epipetalous, G (2); fruit a carcerulus with 4 nutlets, rarely a drupe.

548. Examine given specimen of *Lamium*. The flower is zygomorphic and hypogynous. Note (a) calyx of 5 sepals, gamosepalous; (b) corolla of 5 petals, gamopetalous, two-lipped, the upper lip representing 2 petals and the lower 3 petals; (c) androecium of 4 stamens (2 long and 2 short)

epipetalous, posterior stamen absent; (d) gynœcium of 2 carpels, syncarpous, superior, stigma branched. Style springs from base of ovary (gynobasic). Ovary is four-lobed with one ovule in each lobe.

Note.—Stems square in cross-section and opposite leaves are characteristic of this order.

Describe the flower and its method of pollination.

Record the floral formula, floral diagram, and longitudinal elevation.

A. Style not gynobasic; nutlets with large attachment surface.

Ajuga and *Teucrium* with upper lip of corolla short, stamens 4 didynamous. *Rosmarinus* with upper lip arched, stamens 2.

B. Style gynobasic; nutlets usually with small attachment surface.

Lycopus and *Salvia* with 2 stamens; *Mentha*, *Origanum* and *Thymus* with stamens 4 almost equal and corolla nearly regular; *Lavandula* with stamens 4 didynamous and corolla nearly regular; others, mostly with didynamous stamens and bilabiate corolla, include *Scutellaria*, *Marrubium*, *Nepeta*, *Prunella*, *Galeopsis*, *Lamium*, *Ballota*, *Stachys*, *Monarda*, *Calamintha*, *Satureia*, *Hyptis* and *Ocimum*.

Anatomy. Hairs simple, stellate or branched; glandular short or long with 1 to many-celled heads; also balloon-like oil-glands. Stomata type V or I. Crystals as needles or plates, seldom clustered. Primary cortex collenchymatous; vessel-perforations simple.

Solanaceæ. K (5), C̄ (5), A5 or 2-4, G (2) obliquely placed; fruit a berry or capsule. Stem with perimedullary ploem.

549. Examine flower of *Solanum* sp. Note (a) 5 sepals gamosepalous, inferior, the odd sepal is posterior; (b) 5 petals sympetalous, inferior, alternating with the sepals; (c) 5 stamens, hypogynous, epipetalous; (d) gynœcium of 2 carpels, syncarpous, superior and placed obliquely to the median plane. The ovary is bilocular, showing numerous ovules with axile placentation. Record the floral formula, floral diagram and longitudinal elevation.

A—5 fertile stamens, embryo strongly curved.

Solanææ—ovary bilocular; *Solanum*, *Lycopersicum*,

Capsicum, *Physalis*, *Atropa* (fruit, a berry);
Hyoscyamus (fruit, a pyxidium).

Datureæ—ovary 4-locular; *Datura*.

B—embryo straight or slightly curved.

Cestreæ—5 fertile stamens; *Nicotiana*, *Petunia*, *Cestrum*.

Salpiglossideæ—4 stamens fertile; didynamous in
Salpiglossis and *Brunfelsia*; 2 stamens fertile with
 2-3 staminodes and fl. papilionaceous in *Schiz-
 anthus*.

Anatomy. Hairs very varied, simple, stellate, branched; also glandular short or long of various types. Stomata usually type V, also IIIb and I. Epidermal cells of leaf often with wavy walls. Calcium oxalate crystals sandy, or single or clustered. Perimedullary phloem present, often with bast fibres and containing sandy crystals. Vessel-perforations simple; medullary rays usually narrow.

Scrophulariaceæ. Inflorescence usually a raceme. K (5-4) C (5-4) A4 didynamous, or 2 or 5 \bar{Q} (2) median; fruit a capsule, rarely a berry.

550. Examine zygomorphic flower of *Mimulus*. Find out whether the flower is hypogynous, perigynous, or epigynous. Note (a) tubular calyx with 5 distinct teeth (sepals); (b) corolla with broad tube (sympetalous) and flat lobes arranged in two lips; (c) andrœcium of 4 stamens, 2 long and 2 short, epipetalous (posterior stamen is undeveloped); (d) gynœcium—made up of 2 carpels, syncarpous, superior; stigma is two-lobed and sensitive to mechanical stimulus; ovary bilocular. Cut T.S. ovary and note axile placentation. Describe the flower and its method of pollination. Record the floral diagram and longitudinal elevation. Examine in a similar manner other given specimens belonging to the same family.

I. Pseudosolanææ—5 stamens, all fertile or 1 staminodal, flower regular; *Verbascum*.

II. Antirrhinoideæ—1 stamen absent or staminodal; upper lip of corolla on outside in bud.

Calceolaria C distended; *Linaria*, *Antirrhinum* C personate, spurred or saccate; *Collinsia* fl. papilionaceous; *Scrophularia* C with short upper lip; *Pentstemon* with one staminode; *Mimulus* C ringent A4.

III. Rhinanthoideæ—upper lip of corolla covered by lateral petals in bud; stamens 4 or 2.

Veronica K4, posterior sepal absent, C5 two posterior petals united, A2; *Digitalis* A4; others are semi-parasitic, C with helmet-like upper lip, A4; e.g. *Rhinanthus*, *Bartsia*, *Pedicularis*, *Euphrasia*, and *Melampyrum*.

Anatomy. Hairs various, simple, stellate or branched; glandular short with 1- to 4-celled heads or long with multicellular heads; also scutate glands in group III. Stomata type I; leaf epidermis often with wavy walls. Calcium oxalate crystals not common. Perimedullary phloem absent; vessel-perforations simple.

Rubiaceæ. Inflorescence cymose; K4-5 $\overline{C(4-5)}$ A 4-5 $\overline{G(2)}$ rarely 1-(∞), usually bilocular.

I. Cinchonoideæ—ovules more than one in each loculus.

Manettia K4 $\overline{C(4)}$ A4 $\overline{G(2)}$; *Cinchona* K5 $\overline{C(5)}$ A5 $\overline{G(2)}$.

II. Coffeoidæ—1 ovule in each loculus. *Coffea*, *Psychotria*.

Galiceæ—herbs with leafy stipules, calyx very reduced or absent; *Rubia* C(5); *Galium* C(4) rotate; *Asperula* C(4); *Sherardia* K4-6, corolla funnel-shaped.

Anatomy. Glandular hairs do not occur on the leaf-blade (*cp.* Caprifoliaceæ); glandular shaggy hairs may occur on stipules; also various secretory cavities or glands. Stomata type IV, sometimes with 2 parallel pairs of accessory cells. Crystals of calcium oxalate occur as raphides and sandy crystals, also needles and styloids. Vascular bundles simple, collateral, not bicollateral; scalariform perforations rare.

Caprifoliaceæ. K5 or (5) $\overline{C(5)}$ A5 $\overline{G(2-5)}$.

Examine and compare *Lonicera* and *Diervilla* (*Weigelia*) A introrse with *Sambucus* A extrorse; also compare flower and berry of *Symphoricarpos* with flower and 1-seeded fruit of *Viburnum*. *Linnæa* has 4 didynamous stamens and a 1-seeded fruit. *Adoxa* lateral flrs. pentamerous, apical flr. tetramerous.

Anatomy. Hairs glandular with short stalk and 4- or more-celled head, also scaly in some species. Stomata type I; the absence of type IV should be compared with Rubiaceæ. Crystals various, but raphides rare. Medullary rays characteristic in L.S. (except *Sambucus*). Scalariform perforations in vessels common.

Valerianaceæ. Inflorescence cymose; K absent or developing as membranous or hairy pappus in fruit, $\overline{C(5)}$ A 4-1, $\overline{G(3)}$; ovary 3-celled or 1-celled by abortion of 2; fruit 1-seeded, a cypsela.

Nardostachys. A4 $\overline{G(3)}$, fruit trilocular; *Valerianella* A3,

fruit with 2 sterile cells and 1 fertile cell; *Valeriana* C gibbous, not spurred A3 G 1-celled; *Centranthus* C spurred A1 G 1-celled.

Anatomy. Hairs simple, 1- to many-celled; glandular and short. Stomata type IIIb. Vessels with simple perforations, except in primary xylem, where scalariform perforations occur.

Dipsacæ. Inflorescence a cymose head; K5-4 or (5-4) often with epicalyx of 2 bracteoles, C(5-4) A4 G(2); ovary 1-celled, fruit a cypsela.

Dipsacus K spiny; *Succisa* K pappose C4; *Scabiosa* K5 bristles; *Knautia* K4-5 awns, C4 in outer zygomorphic florets.

Anatomy. Hairs simple, unicellular, or often with multicellular base; also short and glandular. Stomata type I, rarely IIIb with one cell distinctly smaller; often on both sides of leaf. Vessels as in Valerianacæ; bast fibres absent.

Campanulacæ. K (5) C (5) A5 or (5) G̃ (5-2).

Campanuloideæ—anthers usually free, but appressed in bud; fr. usually regular. *Campanula* A free G̃(5-2); *Jasione* A(5) G̃(2) fr. in head; *Phyteuma* A free, but held together by tips of petals G̃(2-3).

Lobelioideæ—anthers syngenesious; fr. zygomorphic. *Lobelia* C(5) bilabiate, A(5) G̃(2).

Anatomy. Hairs simple, usually unicellular, sometimes silicified. Stomata type IIIb or I. Calcium oxalate absent. Inulin is common as in Compositæ; latex vessels occur chiefly in Lobelioideæ, also in *Campanula* and *Phyteuma*. Hydathodes are common. Medullary phloem strands or vascular bundles occur in many genera.

Compositæ. Inflorescence racemose, a capitulum. Ko C (5) A (5) syngenesious G̃ (2) or Ko C3 + 2 Ao G̃ (2); ovary 1-celled, fruit a cypsela. Corolla campanulate and regular, bilabiate (ray florets, etc.) or ligulate.

551. Examine the inflorescence of Ox-eye Daisy (*Chrysanthemum* sp.). Note the flowers are massed in a compact head (capitulum). Cut a V.S. through the whole inflorescence. Note the two kinds of flower: (1) outer, white, zygomorphic ray florets, bilabiate with upper lip aborted; (2) inner, yellow, tubular, actinomorphic disc florets. Note in ray florets: (a) calyx is absent; (b) corolla of 5 petals united to form a narrow tube at the base—three of the petals grow out to form the broad lower-lip in which 3 lobes can be seen; (c) androecium, absent or rudimentary; (d) gynœcium with inferior ovary. Note in disc florets (a) calyx is absent; (b) corolla of 5 united petals (gamo-

petalous); (c) andrœcium of 5 stamens, epipetalous, united by their anthers to form a tube round the style; (d) gynœcium of 2 carpels, syncarpous, inferior. The style is 2-lobed and the ovary is 1-chambered containing 1 ovule. Record for a ray floret and for a disc floret the floral formula, floral diagram and longitudinal elevation.

Examine capitulum of Dandelion. Note the florets here are all strap-shaped (ligulate). Note the pappus of hairs, which aids in the dispersal of the fruit. Dissect out and draw one flower, also one fruit with developed pappus.

For types of styles and stamens, etc., see Textbook (Figs. T 1299-1300).

I. Tubulifloræ—florets all regular or with outer (rays) or all florets bilabiate.

1. Vernoniæ—homogamous; style type III; stamens type 5; *Vernonia*.

2. Eupatoriæ—homogamous; style type II; stamens simple; *Eupatorium*, *Ageratum*.

3. Astereæ—heterogamous (with ray florets) or homogamous; style types VII-VIII, stamens simple; pappus usually setose or coroniform. *Solidago*, *Bellis*, *Aster*, *Erigeron*, *Callistephus*, *Olearia*.

4. Inuleæ—as 3, but style types XII-XIIa; stamens types 10-14, pappus usually setose or paleaceous. *Helichrysum*, *Gnaphalium*, *Antennaria*.

5. Heliantheæ—as 3, style and stamen types also as 3, but pappus often paleaceous or aristate, seldom setose; receptacle paleaceous. *Helianthus*, *Silphium*, *Dahlia*, *Bidens*, *Cosmos*, *Xanthium*.

6. Heleniæ—as 5, but receptacle not paleaceous. *Helenium*, *Tagetes*.

7. Anthemideæ—as 3, but style type V; stamens simple; pappus absent or coroniform; receptacle with or without paleæ. *Achillea*, *Anthemis*; *Chrysanthemum*, *Matricaria*, *Tanacetum*, *Artemisia*.

8. Senecioneæ—as 3, but style type IV, stamens usually simple; pappus setose. *Senecio*, *Doronicum*, *Tussilago*, *Petasites*.

9. Calenduleæ—ray florets ♀, disc florets usually sterile with style type VI; stamen types 3-6; pappus absent. *Calendula*, *Dimorphotheca*.

10. Arctotideæ—as 3, but style type IX, stamen types 4-10. *Arctotis*.

11. Cynareæ—homogamous or with enlarged outer florets; style types X-XI; stamen types 15-16; pappus paleaceous; receptacle often paleaceous. *Echinops* with compound capitulum,

Carlina, *Arctium*, *Carduus*, *Cnicus*, *Centaurea*, *Cynara*, *Carthamus*, *Saussurea*.

12. Mutisiæ—homogamous or heterogamous; style types various; stamen types 10-14; disc florets frequently bilabiate. *Mutisia*, *Gerbera*.

II. Ligulifloræ—florets all ligulate; latex present.

13. Cichoriæ—homogamous; style type I; stamen types 6 and 8. With pappus absent *Lapsana*; with pappus coroniform *Cichorium*; with pappus plumose *Picris*, *Leontodon*, *Tragopogon*, *Hypochaeris* (receptacle paleaceous); with pappus pilose *Taraxacum*, *Lactuca*, *Crepis*, *Sonchus*; with pappus setose brownish *Hieracium*.

Anatomy. Hairs various, simple, 2-armed, branched, stellate or thick and multicellular; glandular short or long; also mucilaginous single or biscriate as on fruit of *Senecio vulgaris*, etc. Stomata type IIIb or I. Calcium oxalate crystals in needles, small prisms, or small octahedral forms; clustered crystals are rare; large single crystals in a few species, e.g. *Kleinia articulata*. Phellogen superficial; simple or rarely scalariform perforations; schizogenous resin or gum canals common; latex vessels in Cichoriæ and *Gundelia*; latex sacs in many genera, also oil glands and other secretory cells; inulin common.

MONOCOTYLEDONES

Gramineæ. Po or 2-3 lodicules, A3 rarely 2 or 6 or more, G1. Fruit a caryopsis.

552. First-year students should do Ex. 553 first; and then examine inflorescence of *Lolium perenne* (Rye Grass). The inflorescence is a compound spike composed of spikelets alternating on its two sides. Each spikelet arises in the axil of a sterile bract (outer glume). Most grasses have an inner glume on the side opposite to the outer glume, but this is not present in *Lolium* except in the terminal spikelet. Each flower on the spikelet is ensheathed in two fertile bracts or paleæ; the outer one is sometimes called a *lemma*.

Each flower shows (a) 2 lodicules (minute hypogynous scales) which may represent the perianth; (b) 3 stamens, hypogynous free, with versatile anthers which contain powdery pollen; (c) gynœcium, consisting of a pear-shaped, superior ovary and 2 feathery stigmas. The ovary has a single basal ovule. The inconspicuous flowers, versatile

anthers, dry dusty pollen and expanded feathery stigmas indicate wind pollination. The fruit is a caryopsis (Fig. T 792). Draw a single dissected flower. Record the floral formula and floral diagram. Examine other specimens (as supplied) belonging to Gramineæ.

Advanced students are advised to use "The World's Grasses," by J. W. Bews (Longmans, Green & Co.), for the classification of this group. See Ex. 81 for vegetative key to some common grasses. Several exceptions occur in the following characters.

I. Bambuseæ—usually shrubs or trees; A6 or 3 or ∞ ; styles 3-2. Fruit a nut, berry or caryopsis. *Bambusa*.

II. Pharææ—spikelets unisexual, monœcious; lodicules 3, A6 or 3-2. *Pharus*.

Spikelets 2- to many-flowered—

III. Festuceæ—glumes shorter than first floret. *Arundo*, *Phragmites*, *Glyceria*, *Poa*, *Festuca*, *Bromus*, *Brachypodium*, *Briza*, *Dactylis*, *Cynosurus*, *Sesleria*, *Melica*, *Triodia*, *Sieglingia*, *Eragrostis*, *Molinia*, *Catabrosa*.

IV. Avenææ—glumes at least as long as first floret, and awn when present usually bent. *Danthonia*, *Avena*, *Deschampsia*, *Corynephorus*, *Trisetum*, *Koeleria*, *Aira*, *Holcus*, *Arrhenatherum*.

Spikelets several- to 1-flowered—

V. Chlorideææ—spikelets sessile or sub-sessile, on one side of rachis. *Eleusine*, *Chloris*, *Cynodon*, *Spartina*.

VI. Hordeææ—as V, but with spikelets on opposite sides of rachis *Elymus*, *Hordeum*, *Agropyron*, *Secale*, *Triticum*, *Lolium*, *Nardus* (spike unilateral by twisting).

Spikelets 1-flowered—

VII. Agrostideææ—glumes usually as long as outer palea (= lemma). *Calamagrostis*, *Agrostis*, *Ammophila*, *Lagurus*, *Pheum*, *Alopecurus*, *Milium*, *Stipa*.

VIII. Zoysieææ—glumes shorter. *Zoysia*.

IX. Phalarideææ—spikelets with 2 empty lemmas or 2 male florets; A6, 3, 4, or 2. *Anthoxanthum*, *Hierochloë*, *Phalaris*.

XI. Oryzeææ—as IX; glumes minute or 0; *Oryza*, *Leersia*, *Zizania*. (Spikelets unisexual. Stamens 6 or more.)

Spikelets with one perfect terminal flower and one male or barren floret—

XII. Arundinelleææ—awn as in IV. *Arundinella*.

XIII. Paniceææ—lower glume small or 0. *Panicum*, *Paspalum*, *Setaria*, *Pennisetum*, *Spinifex*.

XIV. Andropogoneææ—lower glume longer than floret. *Saccharum*, *Sorghum*, *Andropogon*.

XV. Maydeæ—as XIV, but spikelets unisexual. *Coix*, *Euchlaena*, *Zea*.

Anatomy. Hairs simple, unicellular or multicellular; walls sometimes thick. Stomata II or IIIa. Calcium oxalate crystals single or as raphides. Sclerenchyma often developed in connection with leaf veins, giving "girders"; also sub-epidermal and along ridges; motor-cells along furrows in rolled leaves. Sclerenchyma in rings and ridges with siliceous deposits on outer walls in stem.

Cyperaceæ. Po or 6 scales or ∞ hairs, A3 \underline{G} (3) or (2). Fruit an achene. Leaves 3-ranked, sheathing base entire, with no ligule.

Flowers $\text{\textcircled{f}}$. Glumes 2-ranked—*Cyperus* (fruit brown, Po A3 \underline{G} (3)); *Schænus* (fruit black).

Glumes imbricate—*Eriophorum* (P long hypogynous hairs); *Eleocharis* (spikelet solitary P3 to 6); *Scirpus* (P3 + 3); *Rhynchospora* (plant small, nut beaked); *Cladium* (plant large, leaves with spiny margins, nut obtuse).

Flowers $\text{\textcircled{m}}$ $\text{\textcircled{f}}$. Fruit enclosed in utricle, Po \underline{G} (3) or (2). *Carex*.

Anatomy. Hairs rare, except on fruits. Stomata types II or IIIa. Crystals single or as raphides. Sclerenchyma as in Gramineæ.

Araceæ. Inflorescence a spadix with spathe. P3 + 3 or 0 A3 + 3 or fewer to 1, \underline{G} 1 usually. Mostly exotic.

Acorus and *Anthurium* flrs. $\text{\textcircled{f}}$ P3 + 3, spadix exposed. *Richardia* (= *Zantedeschia*) flrs. $\text{\textcircled{m}}$ $\text{\textcircled{f}}$ Po; latex present. *Spathicarpa* $\text{\textcircled{m}}$ Po A synandrous, resembling *Equisetum* sporangiophore; $\text{\textcircled{f}}$ Po A staminodal, \underline{G} 1. *Arum maculatum* $\text{\textcircled{m}}$ Po A2-4, $\text{\textcircled{f}}$ Po \underline{G} 1; latex present; three types of $\text{\textcircled{f}}$ flrs. are present (see "P-l. P. L.," No. 70).

Anatomy. Hairs rare on leaves, simple when present; internal hairs like sclereids occur (cp. Figs. T 85-86); also hydathode glands. Stomata types II or IIIa. Crystals usually as raphides; also clustered in palisade tissue, and in *Acorus* as single crystals in fibres. Latex vessels in some genera, also mucilage and other secretory cells. For *Arum* see "P-l. P. L.," No. 70.

Liliaceæ. P3 + 3 or (3 + 3) A3 + 3 \underline{G} (3).

553. Examine flowers of Wild Hyacinth (*Scilla*). Note that each flower arises in the axil of a bract and has a smaller bract (bracteole) on its stalk. Dissect one of the flowers and note the following organs: (a) perianth, consisting of 6 petaloid segments, 3 segments of the outer whorl overlapping the other 3 which form an inner whorl; (b) andrœcium of 6 stamens, each stamen being opposite one of the segments to

which it adheres ; (c) gynœcium of 3 carpels, syncarpous and superior ; ovary has 3 loculi with numerous ovules. Placentation is axile.

Description of flowers : actinomorphic, complete, hypogynous, with bract and 1 bracteole present. Perianth blue consisting of 6 segments in 2 whorls. Andrœcium of 6 stamens in 2 whorls, each stamen attached at base to perianth segment. Gynœcium of 3 carpels, syncarpous, superior, placentation axile, ovules numerous.

Record the floral formula, floral diagram and longitudinal elevation. Examine similarly other specimens (as supplied) belonging to Liliaceæ.

I. Melanthioideæ—rhizome or corm ; inflorescence terminal. *Nartheceum*, *Veratrum*, *Gloriosa*, *Colchicum*.

III. Asphodeloideæ—usually rhizome with radical leaves. *Asphodelus*, *Funkia*, *Hemerocallis*, *Phormium*, *Kniphofia*, *Aloë*, *Gasteria*, *Haworthia*.

IV. Allioideæ—usually a bulb or short rhizome ; inflorescence a cymose umbel (or a single flower in *Gagea*). *Allium*, *Agapanthus*.

V. Lilioideæ—bulb ; infr. terminal racemose, or a single flower. *Lilium*, *Fritillaria*, *Tulipa*, *Scilla*, *Ornithogalum*, *Hyacinthus*, *Muscari*.

VI. Dracaenoideæ—stem usually erect, woody. *Yucca*, *Dracaena*, *Dasylyrion*.

VII. Asparagoideæ—rhizome sympodial ; fruit a berry. *Asparagus*, *Ruscus*, *Polygonatum*, *Convallaria*, *Trillium*, *Paris* (fl. 4-merous, leaves net-veined).

XI. Smilacoideæ—climbing shrubs ; leaves net-veined ; fruit a berry. *Smilax*.

Anatomy. Hairs rare. Stomata type II or IIIa. Calcium oxalate crystals single or raphides or styloids (e.g. *Kniphofia*). General anatomy very varied (cp. *Allium*, *Aloë*, *Ruscus*).

Amaryllidaceæ. P3 + 3 or (3 + 3) A3 + 3 \bar{C} (3). Anthers introrse except in *Campynema*.

I. Amaryllidoideæ—bulb ; inflorescence a scape.

1. Amaryllideæ—with no corona *Amaryllis*, *Galanthus*, *Leucojum*, *Hæmanthus*, *Crinum*.

2. Narcisseæ—with full corona *Narcissus* ; with corona as short ring or stipular outgrowths *Pancratium*, *Sprekelia*, *Hippeastrum*.

II. Agavoideæ—rhizome ; leaves fleshy in rosette, *Agave*, *Fourcroya*.

III. Hypoxidoideæ—rhizome ; stem with small leaves. Leaves inverted *Alstrœmeria*, *Bomarea* ; leaves plicate *Hypoxis*.

Anatomy. Hairs rare. Stomata type II or IIIa. Calcium oxalate crystals as raphides or single.

Iridaceæ. P(3 + 3) with tube below, A3 + 0 extrorse, $\bar{G}(3)$, styles usually petaloid.

I. Crocoideæ—fls. solitary or few ; plant small. *Crocus*, *Romulea*.

II. Iridoideæ—fls. numerous, several in each spathe ; sub-aerial stem distinct. *Iris*, *Tigridia*, *Sisyrinchium*, *Libertia*.

III. Ixiodeæ—as II, but 1 flr. in each spathe. *Ixia*, *Tritonia*, (*Montbretia*), *Gladiolus*, *Freesia*.

Anatomy. Hairs rare. Stomata types II or IIIa. Calcium oxalate crystals as raphides or single.

Orchidaceæ. Flrs. zygomorphic. P3 + 3, posterior inner segment (= labellum) in front after resupination, A1 or 2 $\bar{G}(3)$. See Figs. T 1344–1350 for floral details. See Rendle's "Flowering Plants" for classification.

I. Diandræ—A2, pollen loose and sticky ; stigmas 3 fused, not sticky. *Cypripedium*.

II. Monandræ—A1 with 2 pollinia ; caudicles when present joined by disc within rostellum or sterile stigma ; fertile stigmas 2 separate and sticky.

1. Basitonæ—anther not falling readily ; caudicles distinct.

Orchis, *Ophrys*, *Habenaria*, *Gymnadenia*, *Aceras*.

2. Acrotonæ—anther easily detached, and falling early ; caudicles absent. This includes most of the cultivated types, as well as some British genera.

Cephalanthera, *Epipactis*, *Spiranthes*, *Listera*, *Neottia*, *Goodyera*, *Vanilla* ; *Liparis*, *Malaxis*, *Corallorhiza*, *Masdevallia*, *Epidendrum*, *Cattleya*, *Phajus*, *Catasetum*, *Coryanthes*, *Stanhopea*, *Dendrobium*, *Cymbidium*, *Odontoglossum*, *Oncidium*.

Anatomy. Hairs rare ; simple or glandular ; rarely as papillate massive trichomes. Stomata usually type II or IIIa, sometimes I ; usually on under side of leaf and lying parallel to the length of the blade. Calcium oxalate crystals as raphides, styloids, also single and clustered crystals. Various thickened cell walls occur in the mesophyll ; sclerenchyma in stem ; velamen (see Fig. T 296) and mycorrhizal fungus in roots.

CHAPTER XLI

REPRODUCTION

SINCE the subject-matter of the corresponding chapter in the Textbook is largely a brief revision of the methods and significance of reproduction, the following revision questions may be considered useful from an educational point of view, as well as from the standpoint of examinees.

554. Compare and contrast vegetative multiplication with reproduction.

555. What is meant by "vivipary"? Write a short account of this phenomenon in at least three different species.

556. Write a general account of the methods used in artificial vegetative propagation, *e.g.* *layering*, *cuttings*, *grafts*, etc.

557. What is a "*graft hybrid*"? Write a short account of the anatomy and cytology of graft hybrids.

558. Distinguish carefully between *asexual reproduction* and *sexual reproduction*, using as examples the types of plants which you have studied.

559. Enumerate, with brief notes on their special characteristics and functions, the various kinds of *spores* in the lower plants as represented by the types which you have studied.

560. What is meant by "heterospory"? Write an account of this phenomenon in relation to the development of the seed-habit.

561. Explain clearly the terms "*sporophyte*" and "*gametophyte*."

562. Compare and contrast the *alternation of generations* as it occurs in the types which you have studied in the Bryophyta, Pteridophyta, Coniferales and Angiospermæ.

563. Write an account of the stages in the development of sexual reproduction from *isogamy* to *porogamic fertilisation*.

564. What is meant by the following terms: *isogamy*, *anisogamy*, *oogamy*, *zygote*, *phenotype*, *genotype*?

565. Write an account of the significance of the *nuclear fusion* which occurs in fertilisation, including some consideration of the stages in the production of the gametes.

566. Distinguish carefully between *pollination* and *fertilisation*.

567. Write an account of the various methods by which *cross-fertilisation* is obtained in the lower plants.

568. Write a connected essay on the mechanisms which normally result in *cross-pollination*.

569. Discuss the relative advantages and disadvantages of *self-pollination* and *cross-pollination*.

570. Enumerate with specific examples the various arrangements which result in *self-pollination*, (a) before cross-pollination can take place, and (b) after an opportunity for cross-pollination has occurred.

571. What is meant by *prepotency* and *self-sterility*? Enumerate examples of each phenomenon.

572. Give an illustrated account of the various types of female gametangia which occur in the lower plants, with some consideration of the advantages and disadvantages of each type.

573. Give a similar account of the types of male gametes and gametangia.

574. Compare and contrast the methods of asexual reproduction in the Algæ and Fungi, with special reference to the habitats of the types considered.

575. Write an account of the evolution of *sex-differentiation*, as illustrated by the types of plants which you have studied.

576. Compare carefully the relative efficiency of asexual and sexual reproduction in the Fungi, and consider this in relation to the specialisation of habitat shown by heterotrophic plants.

577. Write a detailed account of the structure and dispersal of spores (a) in the Bryophyta and (b) in the Pteridophyta.

578. What is the difference between self-pollination and self-fertilisation?

579. Describe precisely what is meant by *apogamy*, *apospory* and *parthenogenesis*, giving definite examples of each phenomenon.

580. Write a detailed account of *meiosis*, indicating as far as possible the significance of each stage of nuclear division, particularly in relation to the results of subsequent gametic fusions.

CHAPTER XLII

THE USES OF BOTANY

THE intimate dependence of human activities and comforts upon plants and plant products is expounded in "What Botany Really Means,"¹ as well as more briefly in the Textbook. The following practical exercises are designed to give the student some detailed knowledge of a few of the many plant products which are used in everyday life. Starches, sugars, proteins and other reserve materials are considered in Chapter XVIII.

Textile Materials

581. Flax (*Linum usitatissimum*). Examine carefully prepared specimens showing the stages in the preparation of linen thread from flax stems. Note the significance of each of the earlier stages with relation to the separation of the "fibre" from the stem. Cut, stain and mount permanently a T.S. of an unretted stem; note the distribution of the fibre bundles, the thick wall and small rounded lumen of each "ultimate fibre."

582. Chemical Sectioning.² Boil a small quantity of flax fibre for a few minutes in 10 per cent. sulphuric acid, using an evaporating basin; dry the fibre between filter papers without washing; heat in an oven at about 60°-70° C., until the fibre becomes deep brown; mount small portions in 10 to 15 per cent. caustic potash; add a cover-slip and remove excess of alkali with filter paper. Observe under L.P. and tap the cover-slip sharply and repeatedly with the tip of a scalpel. Note the "sections" of fibres which are formed; the last stage of manipulation may result in longitudinal splitting unless the tapping is done carefully. This method of chemical tendering may be used for other difficult materials, and sections can be obtained of many other fibres.

¹ By J. Small. Allen & Unwin. London.

² See el Kelaney and Searle, *Proc. Roy. Soc.*, B. 106, p. 357, 1930.

583. Mount a few of the smallest fibres from flax dust or digested flax ¹ in water or chloral hydrate. Note the narrow lumen; the fine, usually oblique cross-lines produced during the beating which precedes scutching; also the acutely pointed ends of the ultimate fibres.

584. Cotton (*Gossypium* spp.). Mount a small quantity of cotton-wool in water, using alcohol to wet the threads if necessary. Note the flattened twisted hairs with thickened edges and large lumens. The twists make spinning possible.

585. Compare and make representative drawings of flax fibres, raw cotton-wool and absorbent cotton-wool hairs after they have been mounted in cuoxam ² for a few minutes. Note the final solution of the cotton-wool cellulose.

586. Hemp (*Cannabis sativa*). Cut a T.S. of one small strand of hempen rope, using pith and the razor directly above a slide with a little water or chloral hydrate; add a cover-slip and examine the fragments for transverse sections of hemp fibres which show, in T.S., a small linear or stellate lumen (*cp.* flax).

587. Examine fibres from digested hemp ³; note the similarity to flax, also the obtuse ends of the fibres and the cross-lines which are often more conspicuous than in flax.

588. Jute (*Corchorus olitorius* or *C. capsularis*). Cut a T.S. of one small strand of unbleached jute twine or of a strand from the textile base of linoleum. Mount as for hemp in chloral hydrate; note in T.S. the polygonal outline and large rounded lumen of the fibre. Test the preparation for lignified walls with the usual reagents.

589. Examine fibres from digested jute ³; note the smooth walls, the absence of cross-lines and the blunt ends of the ultimate fibres.

590. Manila Hemp (*Musa textilis*). Examine twine made from Manila hemp, by the same methods as in Exs. 586–589. Note in T.S. the large lumen, the intercellular spaces and the lignified walls; note in digested fibres the smooth (or wavy when crushed) walls, the gradually tapered acute ends of the fibres and the presence of sub-rectangular sclerenchymatous cells (*stigmata*) which are characteristic of *monocotyledonous* textile materials.

¹ Prepared by heating raw flax for half an hour in 5 per cent. potash.

² See Appendix II.

³ Digested as for flax or by macerating in dilute sulphuric acid with 10 per cent. chromic acid added.

Oil Plants

591. Linseed (*Linum usitatissimum*). Take dry seeds of flax; mount each in a partially split cork of small size and even texture. Cut several transverse and longitudinal sections; mount one in water and about three in chloral hydrate; add cover-slips and warm the second set carefully. Note under L.P. and H.P. the inner walls of the epidermis, mucilaginous and swelling rapidly in aqueous media; test the section in water for mucilage; few-layered parenchyma; single layer of sclerenchymatous cells elongated along the length of the seed; single layer of cells with reddish-brown contents; narrow endosperm with oil and small protein granules; cotyledons with oil and aleurone grains showing globoid and crystalloid. Examine crushed linseed and identify the tissues as far as possible.

592. Coconut (*Cocos nucifera*). Examine a prepared coconut fruit cut in half; note the smooth outer fruit wall, the fibrous middle layer and the hard endocarp, enclosing one large seed. Examine preparations showing the position and structure of the embryo; for illustrations see "P-l. P. L.," No. 171. Cut a thin section of the endosperm of a freshly broken coconut; note the radially elongated cells of the parenchyma and the emulsified cell-contents; look for crystalline fat and large aleurone grains with very distinct crystalloids. Note also the sclerenchyma and vascular strands of the thin brown seed-coat and of the sclerocarp.

593. Almond (*Prunus amygdalus*). Examine a sweet almond in the "shell"; break the sclerocarp open and note—vascular strands of endocarp, pits and sclerenchyma; also the numerous large balloon-cells of the outer epidermis of the seed-coat, particularly near the micropylar end (see "P-l. P. L.," No. 185). Cut thin sections of the seed-coats and note the large cells with thick brown walls pitted around the base; vascular strands with spiral vessels and calcium oxalate crystals clustered or single; small-celled inner epidermis and adherent remains of endosperm with a single layer of cells having beaded walls and containing oil and protein granules. Cut several thin sections of the cotyledons; clear two sections in chloral hydrate and note—oil globules, small protein granules, aleurone grains with crystalloid and sometimes also a rosette crystal of calcium oxalate.

Beverage Plants

594. Tea (*Camellia thea*). Using a pith-holder, cut a series of thin sections of the midrib region of exhausted tea-leaves, which have been immersed in chloral hydrate for at least four days. These sections should be vertically transverse, vertically longitudinal, and also horizontally longitudinal along the under part of the midrib. Mount a selection of the better sections in chloral hydrate and note the spheroidal clusters of calcium oxalate crystals and large idioblasts. Stain a few sections with lignin reagents and construct a drawing of one idioblast showing three dimensions. Mount a few marginal fragments of tea-leaf in chloral hydrate and warm carefully—look for stomata with the guard cells surrounded by three or four narrow accessory cells (type IIIb); thick-walled adpressed hairs with acute solid tips; peculiar marginal teeth each with a blunt mucro or a scar where the mucro has been; also idioblasts in the mesophyll (see Ex. 35).

595. Coffee (*Coffea arabica*). Examine the coffee beans supplied;¹ note the remains of the seed-coat in the furrow on the flatter side. Cut one bean across at right angles to the furrow; split the micropylar portion carefully along the deep groove; note the two small veined cotyledons and the club-shaped radicle. Mount in chloral hydrate and examine in surface view a small piece of seed-coat; note the elongated sclerenchymatous cells with oblique irregular scalariform pits. Cut a thin T.S. of the endosperm; note the irregularly thickened or beaded cell-walls with large pits, also oil, protein and some small starch grains; see Ex. 318*b*.

596. Cocoa (*Theobroma cacao*). Remove the "shell" from a cocoa bean; place the shell fragments in a Petri capsule with 1 square inch of wet filter paper; set aside. Examine the kernel; mount in water a fragment of the soft outer membrane (remains of endosperm); note in surface view the epidermal parenchyma with crystalline masses of fat and occasional calcium oxalate crystals, also long multicellular hairs with brownish contents. These come from the seed-coat and adhere to the endospermic membrane. Using a strong penknife, cut the kernel across; note the convoluted cotyledons. Cut a thin T.S. of the outer part of a cotyledon; mount one section in chloral iodine and

¹ Coffee beans for this exercise should be preserved and softened by storing for at least a week in Dutch pickle (see Appendix II).

note flat epidermal cells with reddish-brown particles, also numerous small starch grains. Mount another section in water and note large colourless masses of fat which melt to globules when heated and yield slowly the other characteristic reactions for fixed oil; also occasional groups of cells containing cocoa-red as amorphous granules; both fat and cocoa-red dissolve in chloral hydrate. Small protein granules may also be found. Examine a T.S. of a moistened shell fragment; note adherent remains of pericarp; outer seed-coat with giant mucilage cells, spiral vessels, and inner epidermis with U-thickenings; inner seed-coat of parenchyma.

Condiments and Spices

597. Mustard (*Sinapis alba*). Dissect a mustard seed which has been soaked overnight in water; note the folded two-lobed cotyledons which enclose the radicle. Prepare a few seeds for sectioning by arranging them on the end of a small cork, in a drop of gum to which has been added about half its volume of glycerol.¹ Allow this to dry, then cut a T.S. of the seed and note—seed-coat with mucilaginous outer epidermis which swells when an alcoholic mount is irrigated with water; large sub-epidermal cells and sclerenchymatous palisade layer. Within this, note a layer of cells containing protein granules and some other parenchyma, possibly of nucellar or endospermic origin. In the sections of cotyledons, note abundant oil, thin-walled parenchyma, protein granules, and myrosin cells which stain with Millon's reagent a brighter red than the protein.

598. Pepper (*Piper nigrum*). Revise Ex. 5; then, using peppercorns which have been soaked in water overnight, cut T.S. pericarp and perisperm. Mount a thin section in chloral-iodine and note in pericarp—thick cuticle, brownish epidermis, sometimes with calcium oxalate crystals, sub-epidermal layer of sclereids, outer parenchyma with occasional rounded oil cells, inner parenchyma with a nearly continuous ring of large oil cells, some reticulately thickened cells and an inner bright layer of cells with pitted U-thickenings. Note also the narrow brown seed-coat (three layers) and the large polygonal parenchymatous cells of the perisperm with densely packed, small starch grains and occasional oil cells.

¹ This gum-glycerol mixture is very useful for sticking labels upon glass bottles, and in other ways.

599. Allspice, Jamaica Pepper or Pimento (*Pimenta officinalis*). Examine whole fruits; note the occasional stalks, the 4 calyx-lobes often broken off leaving a circular scar. Cut one fruit across the middle and note the two loculi each containing a single, very dark brown, plano-convex seed. The oil which gives an economic value to this flavouring material is mainly in the pericarp, but oil glands occur also in the seeds. Cut a T.S. of the pericarp, mount in water, if necessary clear in chloral hydrate, and note the peripheral oil glands, each with a pair of large cells, like guard cells, lying towards the outside; these surround a circular perforation; note also the groups of idioblasts or sclereids each containing one or a few cluster crystals of calcium oxalate. Cut a T.S. of the seed, in pith, and note the numerous sub-epidermal oil glands.

600. Ginger (*Zingiber officinale*). Examine a piece of ginger rhizome; note the depressed stem scars. Cut a piece across with a pocket-knife; note, on a smoothly cut surface, the narrow cortex, scattered vascular bundles and endodermal line with leaf-trace bundles outside. From this surface cut several thin transverse sections; mount one in water and note the general appearance; mount another in chloral hydrate and warm gently. Note oil cells scattered throughout the cortex; also numerous vascular bundles with sclerenchymatous bundle-sheath fibres, two or three large vessels; slightly developed phloem; also scattered throughout both cortex and stele the numerous oleo-resin cells, which can be seen better in moderately thick sections gently warmed with glycerol (pale yellow) or with aqueous potash (reddish-brown). Scrape the cut surface of the rhizome; mount the scrapings in water and observe the starch grains under H.P.; they are oval or pyriform with the hilum near one end and distinct striæ. Some account of other materials will be found in "Plant Products," by S. H. Collins and S. Redington (Baillière, Tindall, & Cox). See also "Foods and Drugs," by H. G. Greenish (J. and A. Churchill).

TRANSPARATION CURRENT AND ASSO- CIATED PHENOMENA— <i>contd.</i>		GROWTH	PERMEABILITY ETC.— <i>contd.</i>	GEOTROPISM— <i>contd.</i>	HAPTROPISM
(117)	138		(390)	(417c)	440
118	(139)	253	(391)	.. d	441
(119)	(140)	254	(392)	418	442
120a	(141)	255	(393)	419	443
(120b)	142	256	(394)	420	296
(121)		257	(395)	421	297
122		258	396	(422)	444a
123		259	397	(423)	(.. b)
124		260	398	(424)	445
(125)		(261)	399	(425)	
		(262)	(400)	(426)	
		(263)	401	(427)	294
		(264)	402A	(428)	451
		(265)		(429)	(452)
		266		430a	453
		(267)	402B	(.. b)	(454)
		268	403	431	(455)
		(269)	(404)	432	456
		(270)	(405)	(433a)	457
			406	.. b	458
			407	.. c	(459)
					OTHER MOVEMENTS

156	160	161	162	163	164	165	166	167	168	169	170	171	172
CARBON ASSIMILATION—contd.													
(186)	187	188	189	190	(191)	(192)	(193)	(194)	(195)	(196)	(197)		
CARBON ASSIMILATION—contd.													
209	210	(211)	(212)										
RESPIRATION—contd.				NITROGEN CYCLE									
303	304	305	306	307	308	(309)	(310)	311	312	(313)	(314)	(315)	(316)
RESERVE MATERIALS													
505	506	507	508	(509)	510	511	(512)	513	514	(515)	516		
ENZYMES													
HEREDITY—contd.				VARIATION									
(474)	(475)	(476)											
HEREDITY—contd.													
VARIATION													
477	478	479	480	481	482	483	484						

APPENDIX I (C)

In order to facilitate the work and to reduce the expenses due to breakages and other damage, each advanced student in this Department pays a Laboratory Deposit of three guineas, and is supplied with a set of apparatus on loan, including the following :—

- 1 nest of 8 beakers, 2,000 to 50 c.c.
- 3 evaporating basins, 200, 100, 50 c.c.
- 3 Petri capsules, 12 × 1.5 cm.
- 6 dozen test-tubes, 15 × 1.5 cm.
- 1 test-tube rack, 6 holes and pegs.
- 1 graduated flask, 100 c.c., not stoppered.
- 1 burette, 25 c.c.
- 2 pipettes, 5 c.c. and 10 c.c.
- 1 funnel, 8 cm.
- 1 opisometer with rubber teeth.
- 1 retort stand with 2 clamps.
- 1 Farmer's potometer.
- 1 thermometer, 250° C.
- 1 set leaf clasps, types A, B, C, D.
- 1 microscope.

APPENDIX II

Reagents, Stains, Tests, etc.

Agar Medium.—Soak 15 grammes of agar-agar in 500 c.c. of water in a conical flask ; plug the flask and sterilise in an autoclave for fifteen minutes at 115° C. Various nutrient media are added in aqueous solution of equal volume as required, placed in plugged test-tubes or Petri dishes and sterilised.

-**Alcohol.**—Ethyl alcohol.

Ninety per cent. dissolves resins, volatile oils and castor oil ; other fixed oils are not dissolved.

-**Alkanet.**—Used in the form of a diluted tincture ; one part of alkanet root macerated with ten parts of 96 per cent. alcohol for a week ; then diluted with an equal volume of water and filtered.

Red with suberin, fixed oils, fats, resins, caoutchouc, and volatile oils.

-**Aniline Blue.**—A 1 per cent. aqueous solution.

Blue stain with callose, which does not wash out with glycerol.

-**Aniline Sulphate.**—A saturated aqueous solution prepared by placing 100 grammes aniline sulphate in a litre of water and shaking at intervals. The solution is acidified by adding 50 c.c. dilute sulphuric acid. More water can be added as the solution is used up, only until all the aniline sulphate is dissolved.

Bright yellow with lignin, suberin and cutin.

-**Braemer's Reagent.**—An aqueous solution of sodium tungstate 10 per cent. and sodium acetate 20 per cent.

Brownish precipitate with tannins.

-**Calcium Chloride.**—(a) As a desiccating agent, fused.

(b) As a pectic acid precipitating agent, a 5 per cent. aqueous solution.

-**Canada Balsam.**—The viscous commercial balsam must be dried by gentle heat until it becomes a hard brittle solid, from which the mountant fluid is prepared by adding just sufficient xylol to produce a viscous fluid.

-**Carbol Fuchsin.**—Dissolve 1 gramme of basic fuchsin in 10 c.c. absolute alcohol and add to 100 c.c. of 5 per cent. phenol in water.

Cell Contents

-**Starch.**—(1) Swells and dissolves in hot water or in aqueous potash.

(2) Blue with iodine ; colour destroyed by strong alcohol, but reappears on dilution.

Sugar.—(1) Yellow to red precipitate on warming with Fehling's solution, either directly or after boiling in dilute sulphuric acid.

(2) Yellow osazone crystals, on cooling, after being heated with phenylhydrazine hydrochloride and sodium acetate in glycerine for at least half an hour in a water-oven.

Inulin.—(1) Crystallises out slowly (in about one week) with absolute alcohol (see Fig. T 431).

(2) Red-brown when crystals are treated with phloroglucin and hydrochloric acid.

(3) Violet with α -naphthol (10 per cent. in 90 per cent. alcohol) followed by strong sulphuric acid and warming.

Mucilages.—(1) Swell and may dissolve in water ; insoluble in alcohol and in glycerol.

(2) Yellow and granular with subacetate of lead in aqueous solution.

(3) May stain with chlorzinciodine, corallin red or methylene blue.

Fixed Oil and Fat.—(1) Pink with alkanet (tincture) and with Soudan red.

(2) Brown to black with osmic acid (1 per cent. aqueous solution).

(3) Not readily soluble in 90 per cent. alcohol (except castor oil). Soluble in caustic potash.

Volatile Oil.—(1) Pink with alkanet.

(2) Soluble in 90 per cent. alcohol.

Proteins.—(1) Yellow or brown with iodine solution.

(2) Red with 10 per cent. solution of mercury in fuming nitric acid (Millon's reagent).

(3) Yellow to orange with nitric acid, especially when followed by potash.

(4) Albumins soluble in water ; globulins soluble in dilute salt solution ; glutelins soluble in dilute alkali or acid ; gliadins or prolamins soluble in dilute alkali or acid, and also in 70 to 90 per cent. alcohol. Each type is insoluble in the media mentioned for the preceding types.

Aleurone Grains.—(1) Crystalloid gives protein tests.

(2) Globoid remains insoluble in dilute potash, but dissolves in hydrochloric acid.

Alkaloids.—(1) Usually a reddish-brown precipitate with iodine and potassium iodide in dilute aqueous solution. Special reactions are used to distinguish the various alkaloids.

Tannins.—(1) Bluish- or greenish-black with ferric chloride solution.

(2) Brown or yellowish-brown precipitate with Braemer's reagent (10 per cent. sodium tungstate and 20 per cent. sodium acetate in aqueous solution).

Resin.—(1) Red with alkanet.

(2) Soluble in 90 per cent. alcohol.

(3) Distinguished from oils and fats by its microscopical appearance as solid, irregular masses:

Caoutchouc.—(1) Pink with alkanet.

(2) Soluble in chloroform.

(3) Insoluble in caustic potash and in 90 per cent. alcohol.

Calcium Carbonate.—(1) Dissolves with evolution of bubbles of gas in acetic or hydrochloric acid.

(2) Dissolves and slowly recrystallises in needle crystals when treated with dilute sulphuric acid.

Calcium Oxalate.—(1) Insoluble in acetic acid.

(2) Soluble with no evolution of gas in hydrochloric acid.

(3) Acts with sulphuric acid like calcium carbonate.

Silica.—(1) Highly resistant to all ordinary reagents.

(2) Unaltered by burning, then dissolving the ash in hydrochloric acid.

(3) Leaves a silica skeleton when the preparation is heated with strong sulphuric acid.

See also other reagents in this Appendix.

Cell Walls

Cellulose.—(1) Blue or violet with chlorzinciodine (zinc chloride with iodine, potassium iodide and water).

(2) Blue with iodine followed by strong sulphuric acid.

(3) Pink to purplish-blue with iodine and calcium chloride.

(4) Dissolves in a fresh solution of cupric oxycarbonate or oxide in strong ammonia (cuprammonia).

(5) Remains colourless with aniline sulphate and phloroglucin.

Lignin.—(1) Brown to yellow with chlorzinciodine.

(2) Deep brown with iodine and sulphuric acid; dissolves on warming in strong acid.

(3) Yellow to brown with iodine and calcium chloride.

(4) Dissolves in Eau de Javelle (an aqueous solution of sodium hypochlorite); the cell wall then gives the cellulose tests.

(5) Bright yellow with aniline sulphate (saturated aqueous solution, preferably acidified).

(6) Scarlet with phloroglucin (1 per cent. in 90 per cent. alcohol) followed by strong hydrochloric acid.

Suberin.—(1) Yellow to brown with chlorzinciodine.

(2) Yellow-brown with iodine and sulphuric acid; insoluble.

(3) Red with Soudan III. (1 per cent. in alcohol and glycerin), Alkanet and Sharlach R.

(4) Dissolves slowly in boiling 3 per cent. alcoholic potash; stains yellow with strong aqueous potash.

(5) Same as lignin with phloroglucin and aniline sulphate.

(6) Green with fresh alcoholic solution of chlorophyll, acting for at least fifteen minutes in the dark.

-Cutin.—As for suberin. Distinguished under the microscope by the highly refractive properties.

Callose.—(1) Dull red to reddish-brown with chlorzinciodine.

(2) Red with fresh, strong solution of corallin red (rosolic acid, sodium carbonate and water).

(3) Soluble in 1 per cent. aqueous potash and in strong sulphuric acid; insoluble in cuprammonia.

(4) Blue with aniline blue, as a 1 per cent. aqueous solution. Callus plate is left blue after washing with glycerol.

See also other reagents in this Appendix.

Chemical Sectioning.—See Ex. 582.

Chlorophyll, Alcoholic Solution.—Freshly prepared from steam-killed green leaves, e.g. nettle, privet, etc., by extraction of the leaves with 90 per cent. ethyl alcohol on a water-bath.

Green with suberin, after acting for at least fifteen minutes in the dark.

Chloral Hydrate.—An aqueous solution of five parts of chloral hydrate in two parts of water.

Used as a clearing agent; when heated it rapidly dissolves resins, protein and chlorophyll, and renders starch transparent and swollen.

Chloral Iodine.—To 50 c.c. of chloral hydrate solution, add five large crystals of iodine; maintain the saturation by adding iodine as necessary.

Used as a clearing agent, combined with starch-staining for minute grains of starch.

Chlor-zinc-iodine. Dissolve 1.0 gramme iodine with 40 grammes potassium iodide in 40 c.c. water; add this solution to 400 c.c. solution of zinc chloride (s.g. 1.8); maintain the saturation by keeping a few crystals of iodine in the stock bottle.

Blue or violet with cellulose; brown to yellow with lignin, suberin and cutin; red to reddish-brown with callose; starch grains are stained blue and swell slowly in the cold solution.

Corallin Red or Corallin Soda. A small piece of corallin (rosolic acid) is added to a few drops of 30 per cent. aqueous sodium carbonate solution, as required.

When freshly prepared this gives a pink or red colour with callose, ligno-cellulose, starch and some mucilages.

Cuprammonia or Cuoxam. Take 1 gramme of copper carbonate and rub this down in a mortar with 20 c.c. water; add gradually with continued stirring 20 c.c. strong ammonia (s.g. 0.880).

When freshly prepared this dissolves cellulose, but does not dissolve callose.

Diphenylamine Sulphate. Used as a 0.5 per cent. solution in concentrated sulphuric acid or in 50 per cent. sulphuric acid. Gives a blue colour (aniline blue) with nitrates, which disappears on dilution with water.

Eau de Javelle. Take 10 grammes of chlorinated lime in a mortar; gradually add 75 c.c. distilled water, triturating thoroughly; add a solution of 15 grammes potassium carbonate in 25 c.c. water; shake together during four days. Filter and store in a cool, dark place. This may be diluted with an equal volume of distilled water for ordinary use.

Dissolves lignin; cell wall then gives cellulose tests.

Eosin. One per cent. solution of water-soluble eosin in water. General stain, useful for aleurone grains.

Erythrosin. (1) A 1 per cent. solution in clove oil may be prepared by rubbing down 1 gramme of dry erythrosin with enough absolute alcohol to make a thin paste, and adding the necessary quantity of clove oil; 100 c.c. is sufficiently accurate.

(2) A 1 per cent. aqueous solution is easily prepared by shaking the dye with water.

Dutch Pickle. Equal parts of glycerol, ethyl alcohol and water.

Fehling's Solution. (Solution A) Seven per cent. aqueous cupric sulphate.

(Solution B) Twenty-five per cent. potassium hydroxide together with 35 per cent. sodium-potassium tartrate in aqueous solution. Equal quantities of the two solutions should be mixed; boiled to check the absence of self-reduction, and then mixed with the test solution.

Red precipitate with reducing sugars; bluish or reddish-violet coloration with some proteins.

Ferric Chloride Solution. Ferric chloride as a 1 per cent. solution in *distilled* water.

Yields green or blue with tannins.

Strong solution of ferric chloride is a 10 per cent. aqueous solution of ferric chloride.

Glycerine Jelly. Best French gelatine 20 grammes and water 120 c.c.; dissolve; add 80 c.c. glycerol also 0.5 gramme phenol; filter through paper while hot.

Guaiacum Solution. Ten per cent. solution of guaiacum resin in 90 per cent. alcohol. The solution should be reasonably fresh.

It should be boiled on a water-bath with a little blood charcoal and filtered, before being used to distinguish peroxidase from oxidase.

Gum-glycerol. Two parts of mucilage of acacia or commercial "gum" mixed with one part of glycerol.

Used for fixing small seeds to cork for sectioning; also for labels on glass bottles.

Indicators. Usually 0.04 per cent. solutions in distilled water or 20 per cent. alcohol. DER and MR neutral are unstable in aqueous solution, but the alkaline forms in aqueous solution may be stocked and portions adjusted to the neutral point as required (see footnote on p. 162). BAN is used as a 0.01 per cent. solution of the hydrochloride in 80 per cent. alcohol.

Phenol red	PR	red to pink	>7.0	yellow	<6.8
Bromo-thymol blue	BTB	blue to green	>6.4	yellow	<6.2
Bromo-cresol purple	BCP	purple to blue	>6.2	yellow	<5.9
Di-ethyl red	DER	yellow	>5.9	pink to red	<5.6
Methyl red	MR	yellow	>5.6	pink to red	<5.2
Benzene-azo- α - naphthylamine	BAN	yellow	>4.8	pink to red	<4.4
Bromo-cresol green	BCG	blue to green	>4.4	yellow	<4.0
Bromo-phenol blue	BPB	blue to green	>4.0	yellow	<3.4

Iodine Solution. Dilute the solution of iodine-potassium-iodide with four times its volume of distilled water.

Blue with starch and amyloid ; yellow or brown with protein, oil-plasm, and ligno-cellulose.

Iodine and Potassium Iodide. Iodine 1 gramme and potassium iodide 0.5 gramme dissolved in 100 c.c. distilled water. Reacts like iodine solution ; also yields a reddish-brown precipitate with alkaloids.

Iodine and Sulphuric Acid. Iodine-potassium iodide solution as above is allowed to act for a few minutes, then the excess is removed with filter paper and a drop of concentrated sulphuric acid placed upon the test-material. Students should wash up all such preparations under the water-tap as soon as they have completed the observations.

Blue with cellulose ; deep brown with lignin ; yellow-brown with suberin.

Iodine and Calcium Chloride. Iodine solution with 10 per cent. calcium chloride added.

Pink to purplish-blue with cellulose ; yellow to brown with lignin.

Lead Sub-acetate Solution. This is the strong Liquor Plumbi Subacetatis of the British Pharmacopœia.

Yellowish precipitates with mucilages.

Loeffler's Methylene Blue. Dissolve 1 gramme of methylene blue in 80 c.c. absolute alcohol and add 100 c.c. aqueous potash (1 in 1,000).

Methylene Blue. A 1 per cent. solution in (a) water, or (b) 90 per cent. ethyl alcohol.

Persistent blue with lignin, suberin, cutin and some mucilages.

Millon's Reagent. A 10 per cent. solution of mercury, dissolved in fuming nitric acid and diluted with an equal volume of distilled water.

Red colour and precipitate with proteins.

α -Naphthol. A 10 per cent. solution in 90 per cent. alcohol, followed by strong sulphuric acid.

Violet with inulin, when warmed gently over a small flame.

Nitron. A 10 per cent. solution in 5 per cent. acetic acid, aqueous solution.

Orcinol. Orcin or methyl resorcin as found in commerce.

With strong hydrochloric acid gives a bluish-purple with pentoses, and a red colour with hexoses.

Osmic Acid. A 1 per cent. aqueous solution, which should be kept in the dark.

Brown to black with oils and fats ; it yields the same colours very slowly with oil-plasm, and may not react at all with palmitin, stearin and some other fats.

Phloroglucin. One per cent. solution in 90 per cent. alcohol ; followed by strong hydrochloric acid in lignin test.

Red with pentoses ; no coloration with hexoses. Red with lignin, suberin and cutin ; reddish-brown with solid inulin.

Acid Phloroglucin. May be used in place of the above. It is prepared by dissolving 1 gramme phloroglucin in 60 c.c. alcohol (90 per cent.) and adding 40 c.c. strong hydrochloric acid.

Picric Acid. Saturated aqueous solution (1 in 90). Stains aleurone grains yellow.

Potash, Alcoholic. (a) Three per cent. solution in 90 per cent. *ethyl* alcohol.

Dissolves suberin slowly, on boiling.

(b) Thirty per cent. solution in *methyl* alcohol is used for chlorophyll separations.

Potash, Ammoniacal. Wash stick potash free from carbonate incrustation ; add a quantity of water which is insufficient to dissolve all the potash, pour off the saturated solution and add to it an equal volume of strong ammonia (s.g. 0.880).

Saponifies fixed oils, producing after a time granules with drying oils and radiate groups of needle crystals with non-drying oils.

Potash, Aqueous. (a) A "strong" solution is made by dissolving about 20 grammes in 100 c.c. water.

Yellow with suberin.

(b) The usual solution is 5 per cent. in water.

This acts as a clearing agent by dissolving oils, proteins, tannins and by swelling starch. It also swells cell walls and is used for disintegrating parenchyma.

(c) A 1 per cent. solution dissolves callose.

Potometer Notes. (1) The use of a *rubber* stopper with three holes is strongly recommended ; sealing is so much simpler and effective.

(2) The leafy twig should be inserted through this stopper by the "cork-borer method" as follows : a twig of slightly greater diameter than the hole in the stopper is selected, cut under water, and kept with the cut end under water until required ; a cork-borer of slightly greater diameter than the twig is wetted and forced through the hole in the stopper from below upwards ; the lower end of the twig is then inserted in the cork-borer from above downwards and the cork-borer withdrawn, leaving the stopper closely applied round the twig.

(3) Sealing mixture should be applied around the twig where it emerges from the stopper.

Resorcinol. Resorcin of commerce, see Seliwanoff's solution.

Sealing Mixtures. (1) For general use a mixture of equal parts of beeswax and soft paraffin is effective.

(2) A harder mixture of two parts of beeswax with one part of soft paraffin is useful during hot weather.

(3) A harder and more adhesive mixture, which requires melting when used, is made with seven parts of beeswax and ten parts of soft paraffin, melted together and mixed with three parts of powdered resin, the last being added gradually to the melted mixture.

Seliwanoff's Solution. Resorcinol 0·05 per cent. in 50 per cent. hydrochloric acid.

Yields a red colour with inulin and levulose (ketone sugar); no coloration with dextrose (aldehyde sugar).

Shellac Varnish. Sticky, for attaching glass indicating filaments to parts of plants, may be prepared from the scaly "orange" shellac of commerce in small quantities as required, by warming a few scales with a few drops of 96 per cent. alcohol on a water-bath; or by allowing shellac varnish to concentrate, by exposing a few drops to the air.

Sodium Picrate Paper. Prepared by soaking strips of filter paper in 1 per cent. or saturated aqueous picric acid solution and drying them. These strips are moistened with 10 per cent. solution of sodium carbonate and used in the moist condition. A red colour is slowly developed in the presence of small quantities of hydrocyanic acid.

Soudan Red or Soudan III. One per cent. solution in equal parts of alcohol and glycerol.

The Soudan III is dissolved in 90 per cent. alcohol and the glycerol is added to this solution.

Red with suberin, fats, also fixed and volatile oils.

Stains

Many sections can be examined more readily when stained red with safranin (1 per cent. in water) or eosin (1 per cent. in water), or reddish-purple with hæmatoxylin. These stains used singly do not differentiate one tissue from another, but, when certain double stains are used, most tissues can be stained a distinctive colour.

For glycerin-jelly staining see Ex. 490.

A combination which is easy to use and gives good general results is safranin and Delafield's hæmatoxylin. Method: Soak in 1 per cent. aqueous safranin for quarter of an hour to two hours; wash in water; soak in hæmatoxylin (aqueous) for four to ten minutes or less; wash quickly in tap water and mount in glycerin jelly, or dehydrate with absolute alcohol, wash with xylol, and

mount in Canada balsam and xylol. All lignified, suberised or cuticularised walls are stained red, also chromosomes and nucleoli. The other tissues are a general purple.

A more difficult method, but one which differentiates much more effectively, is methylene blue (1 per cent. in 70 per cent. alcohol) with erythrosin (1 per cent. in clove oil). Method: Soak in methylene blue for five to fifteen minutes; wash *thoroughly* in methylated spirit; dehydrate very carefully and completely in absolute alcohol (this is the dangerous point: if any water at all is left in the sections they do not counter-stain); soak in erythrosin and clove oil, and watch the counter-staining under the low power of the microscope until it reaches the desired stage; drop on at once a few drops of xylol; wash in xylol and mount in Canada balsam and xylol. All lignified, suberised or cuticularised tissues are stained various shades of blue or green, also tannin (dark blue), chromosomes and nucleoli. Other tissues and cell contents are stained various shades of red, pink or orange. With practice this combination can be used to differentiate as a distinct shade of blue, green, red, orange or purple every single tissue in any section.

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