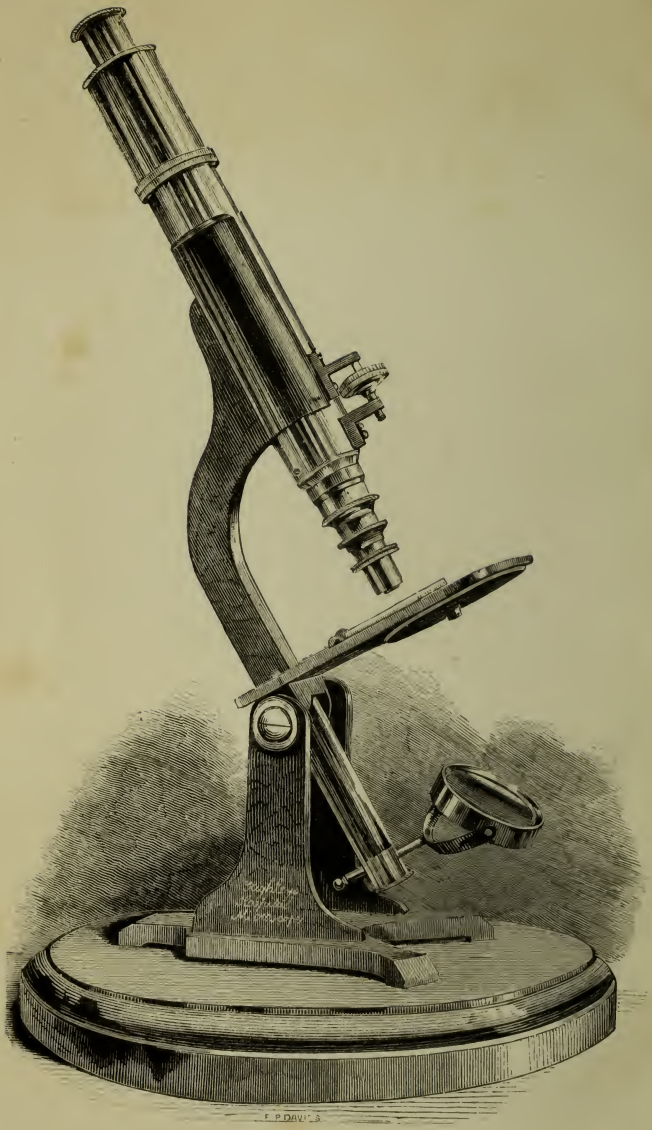

HIGHLEY'S HOSPITAL MICROSCOPE.
From a Photograph.

THE MICROSCOPE,

IN

ITS SPECIAL APPLICATION

TO

VEGETABLE ANATOMY AND PHYSIOLOGY.

BY

DR. HERMANN SCHACHT.

TRANSLATED BY

FREDERICK CURREY, ESQ., M.A.

WITH NUMEROUS ILLUSTRATIONS.

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PREFACE.

THE Work of Dr. Schacht, of which a Translation is now offered to the public, relates to a branch of Microscopical Science which has not hitherto formed the subject of a separate Treatise; and the high reputation of the Author, and the interesting nature of the subject, have induced a belief that the present Version is likely to meet with a favourable reception.

It has been thought advisable to omit the greater part of the description of foreign Microscopes and auxiliary instruments contained in the Original Work. These details would, for obvious reasons, be uninteresting, if not useless, to the English reader. There is no doubt of the superiority of English instruments over those described by Dr. Schacht; and the elaborate and able Treatise of Professor Quekett affords all the necessary information upon the subject of English Microscopes, &c.

The high price of good English Microscopes has hitherto been an impediment to the progress of Microscopy, and much attention has lately been directed to the production

of cheaper instruments. A very useful and convenient form of Student's Microscope is represented in the Frontispiece, which has been designed by Mr. Samuel Highley, jun., of Fleet Street, and may be had at a very moderate price.

The figures of the Original Work, and their descriptions, have been incorporated into the text of the Translation, by which means the inconvenience of constant reference to the plates and their explanation is avoided. The figures of the foreign instruments, and a few other figures, which were not essential for the elucidation of the subject, and which would have increased the expense of the Translation, have been omitted.

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ERRATA.

Page 17, line 15, *for* "oscillatoria" *read* "Oscillatoriæ."
,, 24, line 3, *for* "Hipporchia *read* "Hipparchia."
,, 24, line 2 from bottom, omit semicolon *after* "defined," and insert it *after* "side," in next line.
,, 26, line 18, *for* "holds" *read* "hold."
,, 27, line 23, *for* "salicis" *read* "scalaris."
,, 52, line 6, *for* "Cinclidium" *read* "Cynclidium."
,, 53, line 8, *for* "Haplomytrium" *read* "Haplomitrium;" line 17, *for* "cilia" *read* "ciliæ;" and, line 19, *for* "Rhizocarpeæ" *read* "Rhizocarpæ."
,, 56, line 16, *et seq.*, *for* "Epipogum" *read* "Epipogium."
,, 58, line 10, *for* "Cupressini" *read* "Cupressinæ."
,, 60, line 8, *for* "tuberous" *read* "suberous."
,, 66, line 4, *for* "Callytris" *read* "Callitris;" and, line 9, *for* "Meryolix" *read* "Meriolix."
,, 68, line 12, *after* "Clarkia" *add* "Fuchsia."
,, 71, line 2, *for* "Hallorageæ" *read* "Halorageæ."
,, 73, line 18, *et seq.*, *for* "Ænothera" *read* "Œnothera."
,, 75, line 11 from bottom, *for* "cilia" *read* "ciliæ."

THE MICROSCOPE.

CHAPTER I.

INTRODUCTION.

The progress which has been made in natural philosophy, has been proportional to, and has in fact resulted from, the progress which has been made in the science of optics; by the brilliant improvements in the telescope and the microscope, the philosophy of modern times has made prodigious advances. As the world of great things, the starry heaven, was laid open to the eye of mankind by the telescope, and by means thereof a knowledge of the grandest and simplest natural forces was acquired, so the world of small things and its wonderful governance has been laid open by the microscope. The telescope, as its name implies, is used for exploring distance; it carries us afar into illimitable space, it brings to our notice unchangeable laws which have subsisted for thousands of years, it removes us from the earth, it points out to us other worlds; the microscope brings us back to earth, its utility is confined to our own planet, it is the instrument of proximity, it lays open to us the wonders of small things, it points out to us the innermost construction of the objects which surround us. In the regularity of this construction, the great beauty of which is visible in all organisms, and which we behold with wonder even in the smallest forms of crystallization, we recognise universal laws established by an Almighty hand, although the forces under which these laws subsist are not so well known to us as the laws of the world of space. The knowledge of small

things, and of the influences and changes under which they subsist, is extremely important in many branches of natural philosophy; neither the chemist, the zoologist, the geologist, or the botanist, can dispense with this knowledge; to them the microscope has become an instrument of necessity, an indispensable auxiliary in obtaining information.

But the possession of a microscope, and the perfection of such an instrument, are not sufficient; for useful investigation more than this is necessary. It is necessary to have an intimate acquaintance, not only with the management of the microscope, but also with the objects to be examined; above all things it is necessary to see with intelligence, and to learn to observe with judgment. *Seeing*, as Schleiden very justly observes, is a difficult art; seeing with the microscope is yet more difficult, as it deprives our eyes of all assistance from the surrounding unmagnified objects, and thereby renders any comparison with them impossible. This is a fact which we must not only be aware of, but must constantly bear in mind. In microscopic observation two things must be remembered,—1st. That in the microscope, especially with high powers, we see *surfaces*, not *bodies*. It frequently happens that in looking upon surfaces, we get a glance into the depths of transparent objects by changing the adjustment, without altering the position of the object; it more often happens, however, that in looking upon such objects, we are unable to make them out to be bodies until we have changed their position, and ascertained their dimensions in three different directions; this, in many cases, from the nature of the object itself, is a matter of great difficulty. 2nd. That we seldom see the objects under the microscope in their natural condition; that we consequently must take into consideration the changes which we ourselves partly produce, either by the medium in which the object is placed, or by the use of the knife or other influences. Long and thorough practice with the microscope secures the observer from deceptions which arise, not from any fault in the instrument, but from a want of acquaintance with the microscope, and from a forgetfulness of the wide difference between common vision and vision through a microscope. Deceptions also arise from a neglect to dis-

tinguish between the natural appearance of the object under observation, and that which it assumes under the microscope.

To these difficulties must be added those originating in the eye itself, through the so-called "Mouches volantes," and those also which arise from the observer being unacquainted with the appearance, under the microscope, of the common things which are dispersed throughout the air and water, such as small particles of dust, &c. Lastly, Deceptions are also caused by air-bubbles, by molecular motion, and by the currents which arise upon the stage of the microscope from the evaporation of water, or from the intermingling of two fluids. The observer must learn to know and distinguish all these things thoroughly, and then no further deception can arise from these causes.

The proper use of the microscope is always the principal things to be considered. Hedwig, with the microscope of his time, promoted the advancement of science to a greater extent than many observers with incomparably better instruments have done.

In order to use the microscope properly, the observer must be skilful in handling the instrument and the objects, and, above all things, his mode of proceeding must be conducted with accuracy and judgment, and he must be able to give a sufficient reason for every thing that he does. His progress in research will be slow, but sure; he must endeavour to obtain objects from every possible source, and must examine them thoroughly; he must verify his own observations as scrupulously as possible, and so, progressing step by step, he will attain the desired end. *Work* without *method* will seldom lead to any result; the finest sections of wood made only in one direction, or in a wrong direction, do not lead to any knowledge of the wood under observation. Single observations (of wood, for instance), irregularly made from time to time, only show the condition of the wood at the time of that particular observation, and throw no light on its condition at an earlier or later period; whilst sections made in a proper manner, and well-preserved specimens of the successive conditions of the wood, furnish irrefragable proofs, the one of the construction, and the other

of the development in growth, of the wood under observation. Many a disputed question would have been long since settled, if the proceedings for the purpose of so doing had always been systematic, consistent, and properly conducted. Without perseverance nothing can be effected with the microscope; whilst, on the other hand, by perseverance and determination certain results are to be attained. For the mode of proceeding in general, Schleiden has given several excellent hints, but I know of no guide for research in the field of scientific botany, notwithstanding the numerous writings which exist on the subject of the microscope: such a guide, therefore, appeared to me to be one of the wants of science, and I venture to offer myself in the following pages, as a guide to all those who desire earnestly and zealously to cultivate the science of botany. There will not be found in this book a detailed mode of proceeding for every particular case, inasmuch as I could not possibly, from my own experience, be acquainted with every case, still less, treat fully respecting it; doubtless, like my predecessors, I shall have overlooked many things, and perhaps have entered into many superfluous particulars: but, as far as regards matters of importance, there will be found in this work everything which, after mature consideration, I have thought necessary.

Well-conducted investigations, even if they produce nothing new, but only corroborate previous ones, are of great value; superficial observations give no aid to knowledge. Observations made only for amusement, that is to say, observations of this or that object, made without design or connexion, may be now and then very entertaining, but afford little instruction to the observer, inasmuch as information strung together without method, leads to no real enlightenment. There will not, therefore, be found in this book an enumeration of pretty or amusing microscopic objects, but the collective results of the author's experience in the microscope, as the ground-work for the inquiries of others.

CHAPTER II.

CONCERNING THE NECESSARY APPARATUS FOR THE PHILOSOPHICAL CONDUCT OF MICROSCOPICAL INVESTIGATIONS.

1. The compound microscope.

2. A simple microscope, for dissecting and preparing specimens.

3. A good hand magnifier. 4. A camera lucida.

5. A compressorium, or microscopical crusher. A gentle pressure with the handle of a scalpel, upon the glass which covers the object, may in some cases be substituted for this instrument, which in botanical investigations is comparatively but seldom employed. Where, however, it is wished to observe the changes in the object which take place during, and by means of, the pressure, then such an instrument is indispensable.

6. Razors, scalpels, needles, scissors, forceps, hones, razor-strops.

7. An instrument such as that represented in Fig. 1.* AGHB is a piece of cylindrical brass tubing open at the top, AB, and closed at the bottom; COD is a brass disk fitting the cylinder, and capable of being moved up and down the whole length of the tube by the screw OP, which is worked by the milled head, EPF. Into this tube is pushed a very soft, perfect cork, which has been divided almost to the bottom in the direction QO, that is, longitudinally, with a sharp knife. The object from which it is wished to take a thin section, is placed with great care in the slit made in the cork, and the cork is then pushed

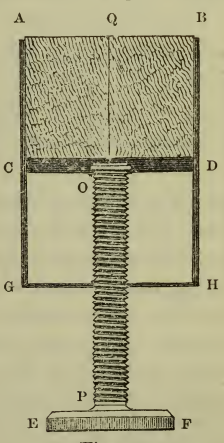


Fig. 1.

* Dr. Schacht recommends a metal tube, open at both ends, and a cork

into the tube so as to leave the upper surface of the cork just projecting above the edge of the tube; the upper surface of the cork is moistened with a little water, and then with a sharp razor, to which the cork itself serves as a guiding surface, the thinnest possible lamellæ of cork are cut from the upper surface; with these lamellæ of cork is obtained a thin lamella of the object placed in the slit of the cork, which lamella is removed from the razor and separated from the cork with a fine camel's-hair brush. This mode of proceeding is strongly to be recommended: it is well adapted for all thin objects, and for all small objects which are not too soft, for example, for transverse and longitudinal sections of leaves, of the stems of mosses, of small seeds, and such like things. If the object is somewhat thicker, the cork must be carefully hollowed out a little at the place which is to receive the object. This plan is useless for very soft objects, which can only be cut with the hand.

8. Some large and small camel's-hair brushes, for the purpose of removing the sections of objects on to the slides. For very small objects, none but the finest water-colour brushes are of any use.

9. Glass utensils, such as small bell glasses (or inverted wine-glasses without legs) for preserving objects from dust, and for collecting the spores of mosses and liver-worts: also watch-glasses, of tolerably large diameter, for treating preparations with water, alcohol, or ether, and for boiling thin sections with chlorate of potass and nitric acid; also long and tolerably wide tubes for warming preparations with water or alcohol, and for boiling objects with chlorate of potass and nitric acid, as has been just mentioned; also the thinnest possible glass rods, for applying small drops of certain re-agents to the object; also glass slides for mounting objects.

divided into two parts. The disk and the screw were suggested by Mr. Ross, who made for me the instrument here described. It is better that the cork should not be quite divided, as there is then less risk of injuring the object by friction whilst pushing the cork into the tube. The screw being well made, the razor very sharp and *flat-sided*, and the cork sound and good, wonderfully thin sections may be obtained with the instrument here recommended.—Tr.

10. Some flat white porcelain saucers (common white saucers are the best). Two at least of these, filled with clean water, must be placed upon the observing-table; one of these serves for immediate use during the observation, the other for the reception of slides and covering-glasses which have been already used. The greatest cleanliness and accuracy is necessary for any regular investigation, and slides and covering-glasses are more difficult to clean, and are more easily scratched, when objects become fastened to them in drying.

11. Some pith of the elder-tree, and some fine linen which has been frequently washed (cambric which has been previously used is the best), for the purpose of cleaning the object-glasses and eye-glasses. Cloths used for cleaning the glasses of the instrument must never be used for cleaning slides and glass covers; for the latter purpose a less fine species of linen may be used.

12. Some chemical re-agents.

i. Alcohol, which is used principally for removing air from sections of wood and other preparations, and as a means of dissolving certain colouring matters, &c.

ii. Ether, which is principally used for dissolving resins, fatty, and other essential oils, &c. This is also useful for removing air.

iii. A solution of caustic potash, which is used for the purpose of dissolving fat, is also useful in certain cases from its effects upon the contents of cells, and upon the thickening layers. This solution often works better after warming.

iv. A solution of iodine (one grain of iodine, three grains of iodide of potassium, one ounce of distilled water) for colouring the cell-membrane, and the contents of the cell.

v. Concentrated sulphuric acid. This is principally used for examining pollen and spores.

vi. Some diluted sulphuric acid (three parts of sulphuric acid and one part water), for colouring the cells of plants which have been previously moistened with the solution of iodine. The object is moistened with the solution of iodine, which is then removed with a fine camel's-hair brush, and by means of a glass rod a drop of sulphuric acid is added,

and the object is then immediately covered with a covering-glass. The effect of the sulphuric acid and iodine, as well as that of the iodized solution of chloride of zinc, is not always the same over the whole surface of an object. At the points where the mixture is more concentrated, the colouring is more intense; frequently places remain without any colour. The colour changes after some time; in twenty-four hours the blue is often changed into red.

The iodized solution of chloride of zinc produces generally the same blue colour in cellulose as iodine and sulphuric acid: the former is preferable in many cases, inasmuch as its effect is not so rapid, and it is not injurious to the cells. Both re-agents should in many cases be employed, and their effects compared with one another. Besides maceration, it is advisable, in examining woods, to adopt the plan of boiling thin sections for about a minute with a solution of caustic potash; after this boiling, the wood-cells, which were not previously turned blue by iodine and sulphuric acid, become of a violet or blue colour upon the application of the iodized solution of chloride of zinc.

VII. A solution of chloride of zinc, iodine, and iodide of potassium. A drop of this solution applied to an object placed in a little water, produces the same colour as iodine and sulphuric acid. This solution was first recommended by Professor Schultz, of Rostock; it is more convenient to use than iodine and sulphuric acid, and produces almost the same results; it is, moreover, not so destructive as sulphuric acid. The exact prescription for this solution is as follows: Zinc is dissolved in hydrochloric acid; the solution is permitted to evaporate, under contact with metallic zinc, until it attains the thickness of a syrup; and the syrup is then saturated with iodide of potassium. The iodine is then added, and the solution, when it is necessary, is diluted with water.

VIII. Nitric acid, or, what is better, chlorate of potash and nitric acid. This is used for separating cells. The method of maceration discovered by Professor Schultz, and which is much to be recommended, is as follows: The

object (wood, for instance) is reduced in size to the thickness of a lucifer-match; it is then thrown into a long and tolerably-wide boiling tube; to this is added, in a little while, an equal volume of chlorate of potash, and as much nitric acid as is at least sufficient to cover the wood and the potash; the tube is then warmed over a spirit-lamp; a brisk development of gas quickly appears; the boiling-tube is withdrawn from the flame, the oxydizing mixture is permitted to work for about a minute and a half or three minutes, and the whole is thrown into a saucer with water: the small pieces which adhere slightly to one another are then collected, placed in the boiling-tube, and boiled repeatedly with alcohol, until the latter appears colourless; they are then boiled once more, for the last time, with water. By the help of the simple microscope the cells are now separated from one another with a needle, and selected. The boiling with nitric acid and chlorate of potash should never be carried on in the room where the microscope is kept, because its glasses might be injured by the evaporation which is developed. Thin sections of plants, for instance, of wood or leaves, are warmed for half a minute, or a minute, in a watch-glass; the boiling is unnecessary in this case; the section is taken out with a little rod, and thrown into a small watch-glass, with water.

IX. Oil of lemons, or any other essential oil, for examining pollen and spores.

X. A tolerably strong solution of muriate of lime (one part of dry muriate of lime, and three parts of distilled water) for preserving microscopic objects. This is useful for most things, even for delicate objects, unless they contain starch. If it is wished to preserve an object for a few days without mounting it immediately, it is a very good plan to put a drop of this solution upon the object, and to place it under a bell-glass for protection against dust.

XI. Glycerine. This is also well adapted for preserving microscopic objects, and especially for cells which contain

starch, which latter substance continues unchanged by it. In granules which exhibit lamination, for instance, in the potato starch, the lamination is apt to continue invisible for the first few hours; after twenty-four hours, however, it appears more clearly.

XII. Copal varnish, or Canada balsam, also for the preparation of microscopic objects; these are only to be recommended for a few thin sections of wood, such as fossil woods. They both make the object more transparent than the solution of muriate of lime.

XIII. Lastly may be mentioned, a tolerably strong solution of carbonate of soda for digesting peat-wood, as well as hydrochloric acid for digesting fossil woods which have been converted into carbonate of lime. Acetic acid, which is often used with advantage for animal tissues, is almost unnecessary for the purposes of botany.

Drawing-paper, lead-pencils, brushes, and colours, are indispensable for proper investigations.

CHAPTER III.

GENERAL RULES FOR THE USE OF THE MICROSCOPE, AND FOR THE ARRANGEMENT OF THE OBJECTS.

One of the principal requisites for microscopical investigation, besides a good instrument, is a proper supply of light. When the position and nature of the apartment can be selected at pleasure, a room should be chosen having windows facing the west or the north, or, what is better, a room with windows towards both those quarters of the heavens. The windows must be as high as possible, since the light received from the horizon is the most favourable; light reflected from a white wall, or the light of white clouds, is often very advantageous.* The light of scudding clouds fatigues the eye by the rapid change in the intensity of the light, besides rendering necessary a continual change in the position of the mirror. No ordinary observation is possible in direct sunlight; this light is, in the first place, far too dazzling for the eye to bear; and, in the second place, it causes appearances which give rise to the grossest deceptions. In working with the microscope in the forenoon and in the middle of the day, a room lying to the east or to the south must therefore be avoided: by means of white blinds, or curtains, the inconvenience may, to a certain extent, be avoided.

Any person who has regard for his eyes should never undertake microscopical investigations at night;† it is true that many objects are seen very beautifully by lamp-light, but this

* Light reflected from a white cloud opposite to the sun is the best that can be had.—Tr.

† This statement should, perhaps, be modified. As long as no inconvenience (such as pains in the eye-balls or weakness in the eyes) is experienced, there is nothing to be apprehended from a moderate use of the microscope at night.—Tr.

light is far more glaring than daylight. When the light is made to pass through blue glass before reaching the mirror, it bears a greater resemblance to daylight, and is pleasanter to the eye. A piece of white ground-glass, fastened in a wooden frame, and placed before the lamp, will have the same effect. By regulating the light of the lamp in this manner, objects already prepared may be shown very well by night, but it is hardly possible to make fine preparations with such an illumination; for exact observation, therefore, the day-time only must be selected. In order to intercept the light of the horizon by means of the mirror, the latter is placed at least three feet from the window, the microscope is turned with the mirror towards the light, and the whole instrument, but especially the mirror, is placed in different positions whilst the observer looks through the eye-glass; the light is, in fact, *sought after*: when the field of view appears clearest and brightest, the object which is to be observed is pushed under the microscope.

When it is wished to examine opaque objects with incident light, the microscope may often be advantageously brought nearer to the window. Since for this kind of illumination a much larger quantity of light is necessary, direct sunlight is sometimes desirable; in the absence of this, the condensing lens is used, by means of which the greatest possible quantity of light is concentrated upon the object. In this kind of illumination, the access of light from below, which would interfere with the observation, is prevented by closing the diaphragm. For objects which are altogether opaque, a background which is white, but not glittering, is often advantageous.

The table at which microscopical observations are undertaken must be sufficiently large, and very firm; it must be so arranged that all the apparatus which is ever wanted shall be at hand. Much time is spared by attention to this, and in microscopical investigations time passes only too quickly; moreover, in a very confined space it is impossible to make effectual preparations with the simple microscope. As the chemist requires a special laboratory for accurate inquiries, so the microscopical observer must at least possess a private work-table, which must be used for no other purpose. Roomy cupboards for con-

taining the different apparatus are very desirable additions to this table. Every object intended for investigation should be examined in the first instance with a low magnifying power, since by that means a far larger portion of the object is seen, and thus a better impression with regard to the whole is obtained. Should the light be too strong, the plane mirror may be used instead of the concave one. When the observer has gained as much information as he can with the low-magnifying power, for instance, one of fifty diameters, or, in some cases, even a less magnifying power, the object-glass is changed for a more powerful one. When the most powerful object-glass has been used, and a still stronger magnifying power is found desirable, then a stronger eye-glass is taken. As a general rule, the eye-glass of lowest power should be used, and, if necessary, the magnifying power should be increased by passing from the object-glasses of lower power to those of higher power; but, nevertheless, for seeing with convenience, and especially for drawing, the use of a powerful eye-glass is often not without advantage. As long as the magnifying power can be increased by means of an object-glass, recourse should never be had to the eye-glass, since both the light and the sharpness of outline of the image are necessarily diminished by the use of a powerful eye-glass, which is not the case in using a more powerful object-glass.

In some cases, it is a good plan to shade with the left hand, the eye which looks into the microscope. When an object is thin enough to be seen with transmitted light, it is first illuminated with light transmitted directly, and is examined with different, and gradually increasing, magnifying powers; should any details of the image remain undefined, obliquely transmitted light is used, which is insinuated into all the different corners of the object. In some microscopes this is attained by turning the stage round its axis; where this arrangement is wanting, the position of the object must be changed by moving it with the hand. Lines always stand out most clearly when oblique light falls upon them at a right angle: where, therefore, a line is suspected to exist, or is only dimly seen, particular attention must be paid to this circum-

stance. In submitting objects to incident light, the same rule generally holds good, and particular care must be taken, by turning either the stage or the object itself, to concentrate the light in all possible directions upon the object. Object-glasses of very high power cannot be used with incident light, inasmuch as the shortness of their focal length prevents the light from falling on the object; in this case recourse must be had to less powerful object-glasses, and more powerful eye-glasses. As a general rule, low-magnifying powers are sufficient when incident light is used.

In most instances, objects are examined under water: it is but seldom, as, for instance, in examining pollen or spores, that it is necessary to observe them in different media, and also when dry. In the case of incident light, water often operates injuriously, especially when the object is not quite covered by it: it is therefore advisable, for certain particular objects, as, for instance, the embryos of grasses, to observe them first without water, and afterwards under water; by placing them under a cover, and adding water with a camel's-hair brush, the object is generally sufficiently and fully immersed. When low-magnifying powers are used, it is not necessary that the objects should be placed under a glass cover, in fact, in many cases where it is wished to have the power of turning the object round, or when it is thought that the object may be improved by any additional cutting or preparation, it is very advantageous not to cover it; when object-glasses of very high power are used, the focal distance is so short, that in order to prevent striking the lens against the object, or dipping it in the fluid upon the object plate, it is necessary to make use of glass covers. When these are used, the fluid in which the object lies frequently becomes lessened by evaporation during the observation, in which case a fresh drop is added at the edge of the glass cover by means of a glass rod, or a clean camel's-hair brush, which may be used when it is wished to add a solution of iodine, or of chloride of zinc and iodine, to objects which are already immersed in water.

When any chemical re-agents are used, whether iodine, caustic potash, or any acid, the object should always be covered with a thin plate of glass; in using volatile acids, such as

nitric acid and hydrochloric acid, too much care cannot be taken. I avoid using them whenever I possibly can. The vapour of sulphuretted hydrogen has a very injurious effect upon flint glass, which is used by some opticians for the under side of the object-glass. The microscope must be carefully protected against gases of this kind, and even against chlorine and such like gaseous matter, on which account, as I have observed before, Schultz's method of boiling the objects with chlorate of potash and nitric acid, must not be undertaken in the room where the microscope is kept.

When the microscope is in daily use, it is a good plan to keep it under a high bell glass,* or an ornament shade. When the day's work is over, I particularly recommend every beginner, before he puts his microscope away, to examine his object-glasses carefully with a magnifying-glass, for it often happens, even to a practised observer, to dip his object-glass in the fluid upon the slide, or to dirty it in some other way; if the glass be only wetted with water, it is of no importance; the consequences, however, may be more serious if the water is permitted to dry upon the lens, especially when the water is impregnated with muriate of lime, inasmuch as, after the evaporation of the water, the lime may stick fast to the glass, and give rise to little scratches in the process of cleaning. When the object-glass has become dusty, or dimmed by atmospheric deposits, it may be cleaned with dry elder pith, care being taken to cut off with a clean razor the surface of the pith which has once been used, and thus to obtain a new surface for the further process; the particles of the elder pith are afterwards removed with a clean camel's-hair brush. If the glass has become wet, it is first carefully dried with a clean linen cloth which has been previously washed—cambric or muslin is the best; and afterwards the elder pith is used. If the glass is soiled by any acid or any other biting fluid, it should be rinsed frequently with distilled water passed through a syringe, and then dried and cleaned in the manner above mentioned. The eye-glasses and the mirror

* Good glasses for this purpose, called in the trade *propagating glasses*, may be obtained at Millington's, 87 Bishopsgate Street Without, and at Cogan's, in Leicester Square.—Tr.

are best cleaned with fine cambric, and also with elder pith. Alcohol and ether should never be used for cleaning the object-glasses, or at least only with great care, since these fluids easily penetrate between the fastening of the lenses, and may reach the cement which unites the crown glass to the flint glass. A lens which has been spoilt in this manner can only be rendered fit for use by a practised optician, who can take it to pieces and put it together again. The more carefully a microscope is preserved from injury, and the cleaner it is kept, the better service it will render, and the longer it will retain its original value.

The greatest cleanliness and accuracy are indispensable for microscopical investigations; it must be laid down as a rule, always to use the cleanest water, in the cleanest vessels, for moistening the slides. Even with this precaution it is impossible entirely to protect the object from becoming soiled with particles of dust. Extraneous things of this kind will not easily deceive a practised observer; a beginner, however, may be easily misled by them. Water which has been left standing should never be used, since it too frequently contains the inferior sorts of animals and plants; and when different objects are examined one after another, fresh water should be taken for every new object, in order that no particles of the objects which have been previously examined, may be mixed with the water upon the slide. Many errors may be traced to a neglect of small precautions of this sort.

In order to be able to recognize extraneous objects as such, it is advisable to gain an acquaintance with those things which, notwithstanding all precautions, cannot always be avoided. To this class of things belong, 1st. Air-bubbles, which, with transmitted light, generally appear, in the form of circles of larger or smaller diameter, with a dark, black-looking rim: with incident light, on the contrary, their rim appears of a white colour. When the object is under a glass cover and in contact with it, the larger air-bubbles frequently assume a very irregular shape; the above-mentioned optical fact is generally, however, by far the best proof of the presence of air, and by it the presence of air may be detected both in and between the cells of plants. 2ndly. Colourless, or coloured fibres of paper,

or of linen, woollen, or silk textures, left behind upon the object-glasses, from the cloths with which they have been cleaned, and also the hairs which have been detached from the brush. 3rdly. Granular particles of dust of irregular shape, which are frequently coloured, and are probably produced by the decay of organized bodies. If it is wished to examine plants or parts of plants, which grow either in or upon the earth, or in water, great attention must be paid to the many organized bodies which are likely to be met with: pains must be taken, by careful observation, to become acquainted with the lower forms of animals and plants: it is necessary, for instance, to be able to distinguish the common forms of infusoria, both those which are provided with siliceous coatings, and those that are not; also with the yeast plant, the different forms of mould, the oscillatoria, and such like things, in order to be able to separate them from the particular object under consideration.

The epithelial cells of the mucous membrane of the mouth are also objects which may deceive the observer. They occur when the brush is drawn through the mouth previously to bringing an object upon the object-plate. It is advisable never to pass the brush through the mouth. When in cutting small objects, the latter are held between the thumb and forefinger, or upon the forefinger alone, it often happens that small fragments of the skin of the finger are cut off at the same time. The observer must learn to distinguish these fragments, as well as the small pieces of cork which he will meet with in sections made between that substance.

The knife sometimes causes deceptions of another kind, when, owing to its not being sufficiently sharp, streaks are left upon the surface which has been cut. In hard woods, such as the wood of palms and of tree ferns, and in very thick albumen, such as that of Phytelephas macrocarpa, this appearance may frequently be observed. The streaks, therefore, must not be taken to be anything belonging to the object, such, for instance, as a layer in the thickening substance. The observer will soon ascertain what the streaks are, if he notice accurately the direction which the knife followed.

Appearances of motion, either usual or accidental, may also

give rise to mistakes, and these must therefore be learnt. Molecular motion is peculiar to all very small bodies, contained in a thin fluid medium; it consists of a somewhat trembling motion of these small bodies; it is frequently seen in the interior of pollen grains; it may be observed still better in certain fluids, for instance, milk, when a small quantity is mixed with water, and placed under the microscope, with a magnifying power of from 200 to 400 diameters. When acquaintance is once made with this phenomenon no further deception can be caused by it. The same result follows from accidental currents upon the object plate, which may take place either by evaporation, or by the mingling of two fluids of unequal specific gravity, or by the dissolving of any salt existing in the fluid. When bodies of small size, and especially round bodies, are examined at the same time as objects of greater thickness, for instance, when the spores and elaters of Liver-worts are examined at the same time as the valves of the capsules, upon the same slide, and under the same glass-cover, the former frequently swim about at first in the water, and care must be taken not to be deceived by them. This motion disappears as soon as the fluid comes to rest. The vibration of the threads of Oscillatoria is, on the other hand, a real motion peculiar to the plant, although it has not yet been explained; the same is the case with the rapid and apparently involuntary movements of the phytozoa of ripe Antheridia, and also with the ciliated spores of Algæ. Moreover, the flowing of the juices of the cell in the cell itself, is particularly interesting, the details of which will be given hereafter.

The "Mouches volantes" belong to that class of deceptions which originate in the eye itself; they are of two kinds. 1st. A slimy secretion from the Meibomian glands; in which case they pass over the field of view of the eye, and are more frequent in the case of persons who are not in the habit of using the microscope.* 2ndly. The shadows of the blood disks passing across the eye in a particular part of that organ; since the ramifications of the blood-vessels are always in the same posi-

* It is not generally admitted that *Muscæ volitantes* are caused by a secretion from the Meibomian glands.—Tr.

tion, it follows that the form of this phenomenon is always the same; it shows an irritable condition of the eye. There is another appearance caused by using too brilliant a light; it manifests itself when the direct light of the sun, or the unsubdued light of a lamp or wax-candle is employed; it consists of spots of different magnitude irregularly scattered over the field of view, which are hardly visible in common daylight; if the eye-glass is turned round, they turn with it, and if the latter is carefully cleaned, they become less; they are caused by dirt upon the glasses, which in very bright light takes the form of spots.

Observations are made less frequently with incident than with transmitted light, but since the latter can only be used for very thin objects, the principal point to be attended to in dealing with opaque objects, is to make such an arrangement of them, as to enable the observer clearly to make out their details. The manner in which the object is divided must be regulated and altered according to the nature of the object itself, and the information which it is wished, by the help of the microscope, to obtain respecting it. Firm homogeneous textures, such as wood, must be treated quite differently from delicate objects composed of different organs, such as buds and blossoms; in the case of wood, it is sufficient to take as thin a slice as possible, cut in a certain fixed direction; in the case of buds and blossoms, attention must be paid not only to the direction, but also, particularly, to the point at which the section is made; it is necessary to exhibit an accurate longitudinal section through the middle of the whole bud or blossom, and an equally accurate transverse section made at different heights, in order to ascertain the arrangement of the organs with respect to one another; moreover, the different parts of the organs must be separated and examined by themselves; in cases like this, and especially in inquiries connected with the development of plants, a dissecting microscope is necessary.

In order to insure success, the kind of knife which is used must be suitable to the object under examination. For wood and hard objects, the best thing to use is a good flat-sided razor, with a broad back. Before cutting, the surface to be operated upon should be moistened each time with a little water; the upper

surface in the first instance should be carefully made smooth with another knife, which may be of inferior quality, and the section should then be made by laying the razor quite flat, and drawing it slowly and steadily towards the person of the operator, without removing it from the surface. After every second, or (at the most) every third cut, the knife must be passed over the razor-strop. The thin slices thus obtained must be taken up with a camel's-hair brush which has been previously dipped in clean water, and placed in a drop of water kept ready upon the slide. For delicate or succulent objects, razors with concave-sided blades are much more advantageous; it is unnecessary to moisten the surface of succulent objects prior to cutting them; in other respects they are treated according to the directions given above. The brush with which the objects are brought upon the slide must never be drawn through the mouth, as otherwise the object will be soiled by the epithelial cells of the mucous membrane of the mouth. Large sections will seldom turn out equally perfect throughout their whole extent. The edges of such sections are generally the most perfect; the size of the sections is of much less importance than the delicacy with which they are made, and the perfect preservation of their cells.

The want of homogeneity in the nature of the texture of some objects, frequently gives rise to difficulties far greater than those which, in other objects, are caused by their minuteness; when, for instance, it is wished to obtain complete and delicate sections, both transverse and longitudinal, through the bark, cambium layer, wood and pith of a dicotyledonous stem, it is impossible to make such sections at one trial, because it will be found that at the points of junction of the different tissues a separation (usually caused by the knife) will take place between the adjoining tissues; it will be necessary in such a case to make many sections, and to choose those which are most perfect. The sharpest possible knife, and slowness and steadiness in making the section, are here especially requisite. As a general rule, it is the best plan to pass from the hard to the delicate portions; a section will sometimes be successful if the knife is placed simultaneously upon the different parts, and in

a direction somewhat oblique to that of the wood-cells, or transverse to the course of the medullary rays. In this, as in many other cases, no fixed rule can be laid down, the observer must make experiments for himself, and shape his course according to the nature of the object. The surface from which the section is to be taken must be kept moistened with water.

Succulent or spongy tissues have generally large cells; it is not necessary, therefore, to have thin sections of such tissues, which are always difficult to make. Delicate animal tissues may advantageously be placed in spirit or pyroligneous acid for some days, provided it is not necessary that the tissues should be examined whilst fresh; but I have found that there is little advantage to be derived from treating botanical objects in that manner. It is a good plan, however, in many cases, to saturate delicate portions of animals and vegetables with thick gum-mucilage, and to let them dry slowly in the air. The double-bladed knife, which has been pronounced to be indispensable in dealing with delicate animal tissues, appears to me almost unnecessary for vegetable anatomy. In using the double-bladed knife, particular care must be taken that the space between the two blades is first filled with water, which is best done by shutting up the knife under water.

In dissecting, different methods must be adopted, according to the magnitude of the different objects; objects of large size may be held with the left hand, or with the thumb and forefinger of that hand; very small, or very thin objects, such as the stems of mosses, thin twigs and roots, leaves, small seeds, and such-like things, may be placed between cork in the manner described at page 5. Small and very delicate portions of objects, which will not bear the pressure of the cork, may be laid lengthways between the thumb and forefinger, without pressing them, attention being paid to the position in which they are placed. This method is very useful when it is wished to divide a small object into two equal parts; for instance, in holding an ovule, it is often the best plan to place it upon the forefinger, and only to use the thumb for the purpose of preventing it from slipping out of its place. It is often advantageous to moisten the finger a little, as the object then

less easily slips about. In cases like this, the section is made very slowly and steadily, the left arm being firmly supported by the table. Sections of small objects thus obtained are examined first without a glass-cover, and with a suitable magnifying power. It is often desirable that the object should be turned over, especially when it is wished to improve it by taking a further slice from it; the side from which the fresh slice is to be taken must be carefully examined, as well as the particular spot at which it is to be made. Very small objects may be laid again upon the forefinger of the left hand, in the manner above described, and a fresh cut attempted, which, although not always, is frequently successful. Before cutting, the magnifying-glass should be employed, in order to satisfy the operator that the object is in a proper position for making the intended cut. If, when the section is sufficiently thin, there still remain portions, the removal of which is desirable for determining the question at issue, the object should be placed under a dissecting microscope, and the objectionable parts removed, if possible, by the help of a needle or a fine-bladed knife.

Observations are sometimes disagreeably impeded by the presence of air, which becomes accumulated in the hairy parts of plants, in the intercellular canals, in the vessels, and in wood; it is best removed by placing the object for a few minutes in a small watch-glass filled with alcohol; when taken out of the alcohol it must be put into water, and then transferred to the slide; when it is wished to examine the cell-contents, in which changes are generally produced by the operation of alcohol, the removal of the air may be advantageously effected by the use of the compressorium, which is permitted to operate continuously upon the object, whilst the observer looks into the microscope. In the absence of a compressorium, the fingers may be lightly pressed against the glass-cover. I would mention, as an example, the ovules of orchideous plants, which are only fitted for observation when the air has been removed from between the integuments and the nucleus of the ovule.

For transferring objects from one fluid into another a very fine camel's-hair brush should be employed; needles and other sharp instruments should never be used for this purpose, since

the object may be easily injured by them. When the object is very small, it will be more easily found if the watch-glass is placed upon a dark back-ground.

The microscope only affords a view of one surface of an object; when, therefore, *bodies* are subjected to examination, it is not sufficient for a correct understanding of them to examine one side only; a transverse section and a longitudinal section, and, in fact, frequently, many longitudinal sections in different determinate directions, must be carefully examined and compared with one another before the observer can be satisfied that he has made out the construction of the body under observation. That which in objects of large size is attained by the help of the knife, is effected, in the case of very small opaque objects, by examining them on different sides. In examining small bodies which are very transparent, as, for instance, the ovules of Orchideæ, or grains of pollen or starch, the adjustment of the microscope is varied from time to time, by which means the upper side of the object is first brought into focus, then the middle (which may be called an *optical section*, transverse or longitudinal, as the case may be), and, lastly, the under-side. The more perfect the object-glass the more exact is the focal plane, and the more sensitive is the instrument to any small alteration of focus, on which account the observer should always keep his hand upon the fine-adjustment screw whilst he is employed upon observations requiring much accuracy. The sensitiveness above mentioned increases, in good instruments, in proportion to the magnifying power.* By a careful adjustment it is often possible to examine an imperfectly-prepared object, as, for instance, a section which is not sufficiently thin, the alteration of adjustment being rendered necessary by the fact, that in a well-illuminated and perfect microscope the eye cannot perceive anything which lies above or below the focal plane.

The accurate adjustment of an object is judged of by the sharpness of delineation of the image. The adjustment is more accurate in proportion to the delicacy and sharpness of the

* It increases also with the angle of aperture of the object-glass.—Tr.

lines seen upon small objects, and also in proportion to the fineness and clearness of the outline, which should be soft, but well-defined. The scales of the *Hipporchia Janira*, a common brown butterfly, are very well adapted for enabling a person to judge of the accuracy of an adjustment; the smallest change of focus causes the transverse striæ to disappear; I recommend, therefore, a careful study of these scales, in order that the observer may obtain accurate ideas both of correct illumination and of exact adjustment; any person who can accurately arrange his illumination and adjustment for these scales will find no difficulty in any other case.

In connexion with the adjustment there are some further optical phenomena, caused, I believe, by refraction, which it is necessary to attend to; one example of which is a feeble, generally yellowish colouring, of the edges of objects placed in a particular focus. These appearances present themselves more frequently in proportion as the magnifying power is increased, and particularly, as it appears to me, when the increase of magnifying power is obtained by the use of a more powerful eye-glass. Large grains of starch, such as potato-starch, are very good objects for exhibiting this phenomenon; according to the adjustment, the edge of these objects will be seen either surrounded with a broad, dark, black rim, or with a narrower and somewhat yellowish rim, or with a soft but well-defined outline. In the latter case, the middle of the grain lies exactly in the optical plane; with this adjustment the grain and the concentric rings of the starch can be best distinguished; the dark rim seen in the other adjustment is caused by the edge not being in focus; the yellow rim is the optical phenomenon above referred to; it is seen with high magnifying powers, and in thin sections, and care must be taken not to be deceived by it. In thin sections of wood, for example, the edge of the thickening substance of the wood-cells is seen, at a certain adjustment, surrounded by a narrow rim of a clear yellow colour; on the outside the thickening substance is bounded by a well-defined shadowy outline (*Schatten-contour*), but the narrow yellowish rim is never sharply defined; on the other side it disappears quite gradually. It is distinguished by

the latter circumstance from a certain lamination or inner membrane of the wood-cell, which, when it is present, has always a clearly-defined outline.

In examining small round bodies, such as pollen-grains, the position of the objects should be changed, by gently pushing the glass cover so as to cause the bodies to roll about; by this means different sides of the objects are seen, and from the different images presented to the eye their true form is made out.

Small objects should never be compressed between two glass slides, that being too rough a method of proceeding; if, however, it is supposed that anything is to be gained by compression, then it is advisable to use the compressorium. When the compressorium is cautiously used, the observer, by carefully watching what takes place, can gain a knowledge of the changes produced by pressure during the time the compressorium is permitted to work. In certain cases, where, for instance, the question is, whether a particular object is a delicate cell or a drop of some fluid, the compressorium may be of service; since, if a cellular membrane be present, it will burst and discharge its contents as the pressure is increased, whereas the drop, whether it be oil, liquid resin, or any other chemical substance upon the slide, will only change its form.

In examining any object, whether animal or vegetable, it is not sufficient to observe the nature, form, and arrangement of the cells; it is necessary also to pay attention to their contents, which, in the case of plants, are different according to the functions assigned to them by nature. It is necessary, therefore, to distinguish—1st. Whether a cell is empty, that is to say, whether it contains air, as is the case, for instance, with perfect vessels and wood-cells; 2ndly. Whether its contents are fluid, with a solid substance contained in the fluid. Another question which arises is as to the nature of the fluid contents, that is, whether they consist of a homogeneous fluid, or of fluids of different consistencies, apparently not intermingling with one another; the manner in which these fluids are affected by chemical re-agents has also to be considered. Lastly, the solid ingredients of the cell-contents, and their physical and

chemical nature, must also be attended to. There are some substances dissolved in the juices of the cell, such as sugar, for example, for which no certain chemical re-agents are known, and yet, perhaps, the red colouring of the contents of ripe pollen-grains, which is often observed to arise upon the application of concentrated sulphuric acid, may be a reaction upon sugar, since Schulz has proved that the effect of sugar and sulphuric acid upon a nitrogenous substance is to produce a red colouring. Gum and dextrine are coagulated by alcohol; the presence of nitrogenous substances is proved, as has been stated, by the use of sugar and sulphuric acid, which produce a red colour, or by a solution of iodine, or of chloride of zinc and iodine, and also by nitric acid, with ammonia subsequently added to it; in these three cases an intense yellow, almost brown, colour is produced. When the presence of oil or resin is suspected, the object should be placed in ether or pure alcohol for some hours, which will dissolve both oil and resin. When the juices of the cell holds any salt in solution, some re-agent must be used which operates upon the salt.

The solid contents of cells consist principally (besides crystals) of starch, inuline, and chlorophyll.* In the case of crystals, their form is frequently sufficient to lead to a decision as to their chemical composition; octohedral crystals, so frequent in plants, as well as the long four-sided acicular crystals called *raphides*, are formed of oxalate of lime. Where the form of a crystal does not afford sufficient information, the use of chemical re-agents is often serviceable; carbonate of lime is known as well by the disappearance of its crystals upon the application of sulphuric acid, as also by the escape of carbonic acid in a gaseous form. In this case it is necessary to observe the instantaneous operation of the acid upon the

* Inuline is a substance resembling starch, found in some of the Compositæ. It differs from starch in not containing so large a proportion of the elements of water, and in being coloured pale yellow or brown by iodine. The name is derived from the plant Inula. Chlorophyll (from *χλωρος*, green, and *φυλλον*, a leaf,) is a substance of a waxy nature, which gives the green colour to plants, and is abundant in the cells of leaves. See 'Henfrey's Outlines,' pp. 12, 13.—Tr.

crystal; this may be best observed when the object is laid in a small quantity of fluid under a glass cover, and a drop of acid carefully brought to the edge of the glass cover by the aid of a thin glass rod; by this means the drop of acid affects simultaneously the whole of the object under examination, and there is time to observe the first working of the acid upon the crystal. I also recommend this mode of proceeding when it is wished to observe the first effect of acid upon a section of a plant saturated with iodine.

Starch is characterized by its assuming a blue colour on the application of iodine; inuline is turned a pale yellow or brown by iodine, and is often not perceptible until iodine has been used; chlorophyll is always green, its grains lose their colour upon being subjected to alcohol; the green colouring matter which covers them appears to be composed of different chemical ingredients from the grains themselves, which, according to Schleiden, consist of a waxy substance. There are many other solid or half solid bodies, which appear in the cells of plants, some with, and some without any definite form, and which, upon the application of iodine, generally assume a yellow or brown colour, but sometimes show no change in their colour; the grains in the leaves of some Liver-worts, of *Jungermannia anomala* and *Alicularia salicis*, are instances of such bodies; the nature of these bodies has not yet been determined. Amongst the substances which are turned brown by iodine, must be reckoned the albumen of the seeds of many plants, for instance the *Rhinanthaceæ*. A large field is here open for microscopical inquiry with the help of chemical re-agents. The iodized solution of chloride of zinc might be of service in the examination of starch. I have made some experiments with genuine West Indian arrowroot: the grains at first assumed a clear brown-violet colour; the lamination was not very distinct; by gently warming the slide over a spirit lamp, a blue colour gradually appeared, the laminæ gave way, swelling quite gradually from the outside towards the interior; the inner part of the starch grains sometimes appeared to remain unchanged after the outer laminæ had already become detached; after a quarter of an hour the colour was violet, all the laminæ

had become more or less detached, the outer ones were only partly distinguishable, and had become transformed into a granular substance of a blue or violet colour.

The use of chemical re-agents, however, is not confined to testing the contents of cells, but is also of great importance in obtaining a knowledge of the nature of the cell-wall; for instance, the presence of cellulose in the cell-wall is ascertained by the blue colour which is produced by applying iodine and sulphuric acid, or the iodized solution of chloride of zinc. After maceration in chlorate of potash and nitric acid, all woody and vascular tissue is turned blue throughout its whole extent, by adding the iodized solution of chloride of zinc; the real intercellular substance and the cuticle, on the other hand, are not turned blue by the latter solution, either before or after maceration. The same effect is produced when sections of plants are warmed with caustic potash and macerated with water; the wood-cells in this case also are blue throughout their whole extent, but the intercellular substance when present is not coloured.

CHAPTER IV.

ON THE DIFFERENT APPEARANCES, FORMS, AND ARRANGEMENTS OF THE CELLS OF PLANTS, AND ON THE METHODS OF PROCURING THEM AND EXAMINING THEM.

The cell is the foundation of all the organs of plants; a thorough knowledge of the cell, therefore, in all its different forms is essentially necessary before any special investigations can be successfully undertaken. A knowledge obtained from books and pictures is not sufficient; it is necessary to become acquainted with the elementary organs of plants from actual observation, and therefore to begin the study of plants with the study of those organs. This chapter contains the most important matters connected with the construction of the cell, together with directions for obtaining objects suitable for observation, and for gaining an intimate acquaintance with them.

To begin with the free Cell.—In the pulp of succulent fruits, for instance, in the ripe currant or raspberry, in the fruit of the snowberry, and also in the leaves of carnations, the cells are so loosely connected together, that it is only necessary to take off small portions of the pulp, or of the parenchyma of the leaves with a knife, to place it in a little water upon a glass slide, and to spread it out upon the slide as thinly as possible. In this case, a quantity of isolated cells will be found, like small sacs, closed on all sides, containing in the currant and raspberry a coloured, and in the snowberry a colourless, fluid. Every one of these cells generally contains an evident nucleus or cytoblast, that is to say, a granular little body of a round or oval shape, which is sometimes sharply defined and transparent, but more frequently not so; in the interior of this nucleus are often seen one or more very small, round, and generally transparent

little bodies, which are called nucleoli. When a detached cell of this kind is subjected to a solution of iodine, the membrane of the cell becomes of a pale yellow colour, whilst the nucleus and the granular mucilage which frequently surrounds it, and which is generally spread over the whole surface of the cell-wall, assumes a brownish-yellow tinge: if the solution of iodine is now removed with a camel's-hair brush in the manner pointed out at page 7, and a drop of sulphuric acid of the proper strength added, or, what is better, if a drop of the iodized solution of chloride of zinc is employed, the membrane of the cell itself becomes of a beautiful blue colour, whilst the nucleus and the granular mucilage retain their brownish-yellow tint. The nucleus is sometimes so transparent that it only becomes visible upon the application of iodine; it is particularly visible in the tissue of orchids, where it is large and sharply defined.

The blue colour assumed by portions of plants, when subjected to iodine and sulphuric acid, has hitherto been always considered as an undoubted chemical reaction upon cellulose; after making many observations during a series of years, I am inclined to think that it is an indication of a certain hydrate of this substance. The effect of iodine and sulphuric acid, for instance, upon very young cells is, in the first place, to turn them yellow, then reddish, and afterwards violet, and lastly (frequently not until an hour after the application), they become blue; whilst old cells, on the contrary, assume this colour instantaneously. Since sulphuric acid probably operates to withdraw water from the cellulose, it appears to me that the membrane of the younger cells contains a larger quantity of water, or at least that the water is more condensed in them, so that the hydratic condition, of which the blue colour is a characteristic, can only come upon them by degrees. The same blue colouring of the cellulose results upon the application of a solution of chloride of zinc and iodide of potassium, and also upon moistening the section of a plant with an infusion of iodine; in the latter case, however, the object must be permitted to dry, and distilled water must afterwards be added. By means of the drying, the water appears to be drawn from the cellulose. Withered cells, on the contrary, are no longer

rendered blue by iodine and sulphuric acid, nor by the iodized solution of chloride of zinc; the cell-wall of the brown parts of diseased potatoes continues brown, whilst the neighbouring cells, which are sound, assume a beautiful blue colour.

The brown colour produced by iodine in the nucleus and the granular mucilage, and the permanence of this colour when sulphuric acid is added, is usually taken to be an indication of the presence of nitrogen; it is more than probable that both the nucleus and the granular inner coating of mucilage (the primordial utricle) contain nitrogen, although this brown colour alone affords no certain indication of its presence; for both withered cells, such as those in diseased potatoes, and also the cuticle of leaves, are made yellow, or brownish-yellow, by iodine, and by iodine and sulphuric acid, without, as I believe, affording any reason for suspecting the existence of nitrogen. I must, however, observe that all these last-named parts appear to have a yellow colour before the iodine is used, and that this colour is only heightened by the application of iodine; whilst the nucleus and the granular mucilage are generally colourless in the first instance. Chemistry, unfortunately, here leaves us in the lurch; we can only positively distinguish certain substances in the contents of cells, namely, starch, inuline, chlorophyll, and certain crystallized salts.

When the student has become sufficiently acquainted with the construction of individual cells, and has satisfied himself that they are little closed sacs containing fluid and solid matter; when he has observed that most essential part of the contents of the cells, the nucleus, which is never wanting in young cells, although often concealed by the granular contents of the cell; when he has informed himself concerning the construction of the nucleus, and has ascertained the presence or absence of nucleoli, and the number of them, if any; when he has observed the relation of the nucleus to the cell, that is, whether it floats freely in the juice of the cell, or is fastened to the wall of the cell, then I recommend him to observe the operation of diluted acid (diluted sulphuric acid or nitric acid) upon the fresh cell; by means of this acid, as well as by alcohol, the inner mucilaginous coating of the cell, which is generally granular, is made

to coagulate; it usually becomes contracted like a closed sac enclosing the solid contents of the cell. Mohl called it the primordial utricle; it is found in all young cells as well as in the cells of all succulent tissue, in the leaves of the Aloe and Agave, in the fresh leaf of the Liver-wort, in the cells of succulent fruits, in the young parenchyma of the bark of the lime-tree, and other plants; in very thick cells it can seldom be discovered.

We will now consider the further development of the cell, and there are three points which must be particularly kept in view, first, the growth, that is to say, the increase in size of the cell; secondly, the degree and manner of thickening of the cell; thirdly, the arrangement of the cells with respect to one another.

The cell, which at first always assumes the form of a small closed sac, of a more or less round shape, afterwards increases in size in different ways. Sometimes it increases equally on all sides and at all points of its periphery. In this case the cell, if it is only slightly pressed upon by the neighbouring cells, retains its original round form. This form of cell is of comparatively rare occurrence: it is found in the reproductive cells, in spores and pollen grains, and also in the pulp of ripe succulent fruits. Cells of the kind now under consideration (that is, cells which increase equally on all sides and at all points of the periphery), are more frequently of a polygonal form, owing to the mutual pressure of the cells; the number of the angles of the polygon depends upon the number and arrangement of the cells which are in contact. Such cells, when cut transversely, frequently appear to be pentagonal, hexagonal, or polygonal. Tissue consisting of such cells has been denominated regular parenchyma. It is very widely distributed; it is found in the potato, in the pith of most trees, in the roots of orchids, in the leaves of aloes, and in other plants.

The second mode of development of the cell is that in which it increases to a greater extent in one direction than in another; by this means we obtain cells extended either in length or in breadth. Length and breadth must here be considered only with reference to the arrangement of the cells amongst one

another; wood-cells may be taken as examples of cells extended lengthways, because they follow the direction of the length of the stem; the medullary rays are instances of cells extended breadth-wise, because they run in an opposite direction. In the stems of succulent plants, as, for example, in the stems of balsams, we find the so-called elongated parenchyma, consisting of rather large, slightly thickened cells. The cambium of dicotyledonous plants also contains narrow elongated cells with very delicate walls. The wood-cells are generally much extended, much thickened, and pointed at both ends. In order to examine fully the form of all these cells, the method of maceration recommended at page 8 may be advantageously employed.

The third mode of growth is that in which the cell increases equally on all sides, but not equally at every point of its periphery. The most elegant form of this kind of cell is the stellate tissue, such as that which is found in the pith of Juncus conglomeratus, and in the leaf-stalk of Musa; it is also found, although less regular in its shape, in many other spongy tissues. In the latter case it is often difficult to make out the particular form of the cells. If their partition walls are very delicate, the iodized solution of chloride of zinc, or iodine and sulphuric acid, by which these cells are generally turned blue, is very useful. Tissue of this nature is traversed by air-passages, which arise from the circumstance of the cells only touching one another at certain spots, frequently of very small extent, and from this circumstance also arises their spongy nature.

The increase in thickness of the cell appears to arise from the deposit of solid substances in the interior, upon the original cellulose of the cell-wall. This deposit of new matter frequently appears to take place in the form of a spiral; in very young wood-cells, for instance, in the youngest cells of a fresh twig of Pinus abies, a most delicate spiral band may be observed in spring and in summer: in the older cells it can hardly be perceived. The band which is seen in spiral cellular tissue, the markings in the thickening layers of the liber-cells of Vinca minor, the arrangement of those spots, the thickness of which is less than that of the rest of the cell, and which

occur in the thickening substance of the wood-cells of Caryota urens and Hernandia sonora, as well as the disposition of the slit-shaped pores of many wood-cells (those of Cycas, for example), all afford arguments in favour of the thickening substance being deposited in the form of a spiral. In almost all cells of great thickness, for example, in many wood-cells, there may be seen, besides, a manifest lamination in the thickening substance, from which it would appear as if the thickening took place periodically; later observations upon the wood-cells of Caryota urens and Hernandia sonora have convinced me that, contemporaneously with this lamination, the direction of the spiral may also be changed. The existence of the spiral band shows that the thickening substance is not spread equally over the whole wall of the cell; the thickening substance does not consist of spiral filaments; the spiral only points out the spots where the layers are of greater thickness.

Certain spots may be observed here and there, the thickness of which is less than that of the rest of the cell; these thin spots often occur at very regular intervals; they are frequently arranged in the form of a spiral, and are often of a regular shape; at the places where these thin spots occur there appears to be a change in the substance of the cells. Even in cells of slight thickness the thin spots can be seen upon making thin longitudinal or transverse sections, especially by using the iodized solution of chloride of zinc, or iodine and sulphuric acid; for it will then be seen that these spots continue colourless, whilst the thicker portions of the cell-wall become of a beautiful blue colour. I would mention, as examples, the starch-bearing tissue of the potato, the cells of the leaf of Fegatella conica and Preissia commutata, &c. In cells of greater thickness these thin spots appear as pores, or canaliculi. With the exception of the cuticle of certain plants, such as Cycas revoluta, Aloe succotrina, Hakea, &c., these pores only occur at those points where two cells touch one another, in which case the pore of one cell exactly meets the pore of another cell, but each is divided from the other by a thin membrane (an excellent example of this is to be seen in the albumen of the seed of the date). Between the walls of the two cells

there is often to be seen a lenticular cavity. The wood-cells of the Coniferæ afford the best instances of this, and the vessels of the tropical twining plants, such as Büttneria and Porana, in which branched canaliculi are often to be found, are well worthy of examination. The tissue in which these canaliculi and lenticular cavities occur is called pitted tissue. In order to become acquainted with the construction of the pits, they must be seen from three sides. In Pinus sylvestris, when a transverse section, or a longitudinal section at right angles to the medullary rays, is taken, the pits are seen sideways; the canaliculus of each wood-cell may then be seen, and, between the cells, the lenticular cavity. If a longitudinal section is made in the direction of the medullary rays, the pits are seen from above in the form of two circles, one within the other; the larger circle is the boundary of the lenticular cavity, the smaller circle within it points out the situation of the canaliculus. The latter does not always appear in the form of a circle; in the wood-cells of the Cycas it is in the shape of a slit. When the canaliculus, as is the case in some specimens of wood, runs into the cell in a conical form, a third circle is seen.

Parenchyma and prosenchyma are distinguished from one another by the extent to which the thickening takes place; the former consists of slightly-thickened cells, not pointed at the ends, and not overlapping each other; prosenchyma consists of cells with pointed ends, whose walls are of considerable thickness, and which overlap one another. Nearly allied to these are the liber-cells, whose thickening substance, however, is less firm and brittle, but more pliant and tough; a property which renders them of great utility.

Vessels are distinguished from the other cells of plants by the fact, that they are placed in rows one above the other, and are rendered continuous by the obliteration, in whole or in part, of their transverse partition-walls. When the partition-walls of vessels meet one another in a horizontal direction, the wall is generally found to be perforated by a round hole; this is the commonest case. When, however, the walls meet one another in an oblique direction, a scalariform partition-wall is

often to be found instead of the round holes; that is to say, a wall with slit-shaped cavities lying near one another, and running in the direction of the breadth of the vessel. In the vessels of Alnus, Thea Bohea, Caryota urens, Ephedra, &c., a double row of round cavities generally appear under similar circumstances in the place of the slit-shaped cavities. Real holes in the substance of the cell-wall are sometimes, but very seldom, met with; as, for instance, in the leaf and stem of sphagnum.*

The spiral vessel is the true type of vascular tissue; in it the thickening substance is developed in the form of a continuous spiral band: in the annular vessels, on the contrary, it would appear that the individual coils of the spiral are, by an excessive elongation of the cell, separated from one another, and thereby assume the form of rings. In the stems of balsams, the most beautiful transition from the spiral to the annular vessel is to be seen. In the scalariform vessels which occur in the wood of the vine, in the leaf-stalks of ferns, and particularly in the stems of tree-ferns, the coils of the spiral are, as it were, joined together by a border; such border occurring at those spots where the vessel meets with several of the adjoining cells. In delicate thickened reticulated cells these borders, which unite the coils of the spiral, frequently occur in greater numbers. In the stems of balsams, all the above-mentioned forms of vessels, except the scalariform vessels, may be found in the greatest perfection. The most beautiful pitted vessels are to be found in the wood of Laurus sassafras; the pits and the spiral bands are both present in Tilia Europea; and the same is the case with the wood-cells of Taxus. Schulz's method of maceration is highly to be recommended for the examination of cells. All succulent stems are particularly well adapted for making observations on the vascular tissue.

The vessels, the wood-cells, and the liber-cells, become filled with air as soon as they are completely formed. The contents of the parenchyma, on the other hand, are fluid, and solid

* The apparent perforations in the cells of sphagnum have been proved to be *real holes,* by observing the passage of animalcules in and out of the cells. See 'Quekett's Lectures on Histology,' p. 9.—Tr.

matter appears to be held in solution, or suspension, in the fluid. If a longitudinal section of moderate thickness is made through the bundles of vessels of a fresh portion of a plant, and treated under water, they will appear to be white, if examined with incident light, before the water has time to force its way into the vessels; if they are examined with transmitted light, they will appear black: these appearances are well known to indicate the presence of air. If the section is now placed in alcohol in order to drive the air out of the vessels, and it is brought again under the microscope in a drop of water, so that the vessels become filled with water, they will then appear transparent like the cells of parenchyma.

It will have been seen that the cells of plants vary much in their mode of growth, and in the manner in which they increase in thickness. They are joined together by a secretion, which originates in the cells themselves, and passes into the intercellular substance. When thus joined together they form different sorts of tissue, which we may divide into three different species. 1st. Parenchymatal tissue; 2nd. Woody tissue; 3rd. Epidermal tissue.

1st. Parenchymatal Tissue.—This is characterized by thin-walled cells, the forms of which, however, may be very various; regular parenchyma, consisting of nearly round cells, or of cells whose length is the same as their breadth, is to be found in the pith of most trees; *elongated* regular parenchyma, on the other hand, is to be found in the cambium of dicotyledonous plants; stellate parenchyma is found in the pith of rushes; spongy parenchyma in the air-passages of many aquatic plants, &c. The tissue of lichens, as well as of the higher fungi, may also be considered to be parenchyma.

The reproductive tissue of plants consists of parenchyma. In the cells of the parenchyma new cells are produced, as well as different sorts of vegetable matters, such as starch, inuline, essential and fatty oils; crystals are also secreted in them. Woody parenchyma forms a sort of transition from parenchyma to woody tissue; its cells are somewhat more thickened, but they have not pointed ends, nor do they overlap one another like the proper wood-cells. Parenchyma of this nature is

found especially in the wood of Leguminosæ; in Spartium and Ulex its cells are provided with a delicate spiral band; it is also to be found in the leaf-stalks of ferns of temperate climates, in the stems of herbaceous plants, &c.

2nd. Woody Tissue.—This tissue consists for the most part of true wood-cells, between which, in dicotyledonous plants, vessels and medullary rays take their course. The wood itself is distinguished by lengthened cells of great thickness, which have pointed ends and overlap one another, and which, in their perfect condition, contain air. It is found well developed in the stems of dicotyledonous trees, in the woody fibres of the vascular bundles of palms, and in the woody fibres of the stems of tree-ferns; in both the latter cases the wood-cells are generally of a brown colour: the thickening layers may here be observed very clearly.

3rd. Epidermal Tissue.—This tissue is very various and may be subdivided into the epidermis proper, the epithelium, and the cork. The epidermis proper consists, for the most part, of a stratum of tolerably thick-walled cells. The form of these cells themselves is very various in different plants: in the monocotyledonous plants, such as the grasses. the Irideæ, the Orchideæ, &c., the cells are elongated, and of regular shape; in the leaves of ferns, on the contrary, they are very irregular in shape, being united together almost in a stellate form. In the leaves of dicotyledonous plants they are differently formed in different plants. It not unfrequently happens that the under side of the same leaf has a differently formed epidermis from the upper side. Between these epidermal cells, but more frequently close underneath them, are situated the stomata. With the exception of the Marchantia, the stomata are almost always formed of only two cells. In the Cycas and some Proteaceæ both these cells lie very deeply buried under a crater-shaped hillock formed of many epidermal cells. In the Nerium Oleander they lie in clusters in deep cavities of the leaf formed for the purpose, whilst the smooth upper surface of the leaf has no stomata. The stomata, especially in plants which grow in the air, are generally to be found on the under side of the leaves. In Cycas and Nerium, for instance, they

are altogether wanting on the upper side. In the floating leaves of aquatic plants, such as Hydrocharis and Nymphæa, they are to be found only on the upper side. The epidermis is frequently clothed with hairs; these hairs are generally prolonged cells of the epidermis itself. The hairs may consist of one or more cells; in the latter case they frequently end with a cellular knob like the glandular hairs of Pinguicula vulgaris and Polycarena capensis; the stinging hairs of the Urticeæ, on the other hand, consist of only one cell, the very small end of which bears a little knob which is somewhat bent and very easily broken. The scales of Elæagneæ, of certain Bromeliaceæ, &c., belong also to this class; they are, as it were, compound hairs. Branched hairs, not compound, but rather consisting of a single cell, are comparatively of rare occurrence; they are found in certain species of Alyssum, and are even more beautiful in certain Amaranthaceæ, for instance, in the leaves of Alternanthera axillaris.

The epidermis proper, and the parts belonging to it, such as the hairs and the outer side of the cells of the stomata, are clothed with a continuous covering, the product of a secretion of these cells, which is called the cuticle, and which, in my opinion, covers the whole epidermis, although it is not of equal thickness at every point of it. In the young epidermal cells this cuticle is very slightly developed: it is often, in fact, nearly fluid, and afterwards appears as a firm membrane capable of resisting the strongest sulphuric acid. It is particularly beautiful in the leaves of Cycas, in the stem and leaves of Viscum, in the leaves of the species of Aloe and Agave, and is fully developed in glaucous leaves of a fleshy or leathery consistency. A very careful examination is necessary in every case to distinguish between the true cuticle and the thickening layer of the epidermis. In the Aloe, the greater part of the so-called cuticle is formed from the cuticular layers of the epidermal cells, and under these layers there lies a real secretion which is the true cuticle. The same may be seen more beautifully in Gasteria obliqua, Viscum, and Phormium tenax. In examining the cuticle, a thin transverse section should be warmed in a solution of caustic potash.

The epidermis covers the leaves and the stem of the higher orders of plants; in the lowest plants, such as the Fungi, Algæ, and Lichens, it is altogether wanting; in the Mosses, it is found in the capsules, in the Marchantiæ on the upper side of the leaf; in the Anthoceros it is to be seen on the capsule covered with very beautiful regular stomata. It is present, as has been already observed, in the higher cryptogams. The young branches of trees are always covered with an epidermis; at a subsequent period a layer of cork is frequently formed underneath it, which splits off and carries the epidermis with it.

The epithelium is an epidermis without stomata and hairs: it often consists of papillose cells which frequently secrete a fluid. Epithelium of this nature is seen beautifully in the stigma, in the canal of the style, and in the ovary of phanerogams; the velvety surface of many different kinds of petals, such as the petals of roses, consists of a tissue of this nature. The epidermis of the roots and rootlets which has no stomata, but which, however, is clothed with hairs, is something of the same nature. Schleiden calls it Epiblema.

The cork consists of many tabular layers formed, for the most part, of thin-walled cells. The layers of cork, when perfect, contain, like the wood vessels, nothing more than air; it is frequently, perhaps periodically, sloughed off with the layer of bark to which it is attached, and it is formed anew from the new layer of bark. It is beautifully developed in some species of Maples, in the Ulmus suberosa, in the Quercus suber, &c.

Besides the parenchymatal, woody, and epidermal tissues, we have to consider the vascular bundles, the tissue of the liber, the intercellular passages, and the laticiferous vessels. The elongated thin-walled cells, called the cambium cells, are the most essential part of a vascular bundle. Perfect vascular bundles consisting only of these cells are sometimes, though rarely, met with; in the creeping root and in the runners of Epipogum Gmelini a slight indication of vessels is sometimes, though very rarely, to be seen; these vessels appear first in the stem and in the parts of the flower. Where a new vascular bundle is formed, for instance, in the embryo of phanerogamous

plants, it consists, in the first instance, only of cambium cells, some of which, at a subsequent period, become developed into vessels. The position of these cambium cells regulates the growth of the vascular bundle, as well as the nature of the growth of the plant itself. In the dicotyledonous vascular bundle the cambium layer is situated on the outside, that is to say, is turned towards the periphery of the stem; the cambium here meets with no impediment in its outward growth, it is capable of forming new wood on the inside and new bark on the outside, and by this means the stem is enabled to increase in circumference. Schleiden calls this the free vascular bundle, in contradistinction to the closed vascular bundle, that is, the vascular bundle surrounded by wood-cells.

The closed (or definite) vascular bundle is peculiar to monocotyledons and the higher cryptogams; in these the cambium cells are surrounded by thickened cells; the vascular bundle cannot, therefore, increase in circumference, it only grows at its apex; on this account the stems of monocotyledons and cryptogams do not increase in thickness, they grow only at their summits, and not in their circumference.

The growth of monocotyledonous vascular bundles deserves to be more fully examined than has hitherto been done; according to my latest observations these vascular bundles are, as it were, branched, like the vascular bundles of dicotyledons. I would mention as an example the flower-stalk of the Epipogum Gmelini, the vascular bundles of which proceed, as it were, like branches out of the simple central vascular bundle of the root, and afterwards become still more branched; the same thing may be observed in the stem and flower-stalk of Goodyera repens.

Besides the cambium cells, which are never wanting, the vascular bundles generally contain *vessels* and *wood-cells*. Vessels are cells placed one above another, whose transverse partition walls are broken through, and which contain air. The arrangement of the vascular bundles of dicotyledons can only be studied at the apex of a young shoot, where each newly-formed vascular bundle can be distinguished separately. They may be studied beautifully in Viscum, Tilia, and Pinus. In Palms the

cambium layer is situated between the large vessels and the woody fibres, which are generally much developed; in Ferns, it surrounds the vessels, but is itself surrounded by a more or less strongly developed ring of wood.

Vascular bundles are never formed in the bark; they pass, however, from the stem through the bark into the branches and leaves; if, therefore, a horizontal section be made through the bark, the vascular bundles appear to be cut through in an oblique direction.

The bundles of the liber never originate in the proper wood. They always consist of elongated flexible cells, generally much thickened; they never have any cambium, nor vessels, nor wood-cells. In Viscum, liber-cells are found dispersed even amongst the woody fibres. In dicotyledons they are produced from the cambium as portions of the bark. In the bark of Palms are to be found wood-bundles without any cambium layer, or vessels; precisely similar bundles are to be found, though less frequently, in the interior of the stems of Palms. The liber-cells are to be found, both isolated and in numbers, in the leaves, and sometimes also in the pith of the stem, &c. In the Apocyneæ and Asclepiadeæ liber-cells are to be found which contain a milky juice. In the bark of certain species of Cinchona, and in many other species of bark, are to be found short, capacious, very thick cells, which may be called liber-parenchyma by analogy to that which is called wood-parenchyma.

At the points where many cells meet there are frequently to be found between the cells, chasms, filled sometimes with air, and sometimes, though less frequently, with fluid. These are called the intercellular spaces; they are to be seen beautifully in a transverse section of the leaf stalk of Cycas revoluta; they are also to be found in most parenchymatal tissue, such as the pith of most trees. These intercellular spaces form, as it were, continuous air-passages surrounding the cells, which air-passages seem to debouch into the breathing pores underneath the stomata.

The laticiferous vessels form a continuous, net-like tissue spread round the cells, and consisting of ramifying canals, in

which a milk-like and frequently coloured juice, is contained; it is not yet settled whether they are formed by the cells or by the thickening of the walls of the intercellular passages. They are to be found in the leaf of Chelidonium and Euphorbia. The so-called laticiferous vessels of the Euphorbiaceæ (Euphorbia antiquorum, and E. splendens), appear, according to the latest observations which I have made, to be no other than branched liber-cells. They originate and comport themselves in a manner precisely similar to the laticiferous liber-cells of the Apocyneæ and Asclepiadeæ (Vinca, Hoya). The same is the case with Ficus elastica. If it be admitted that there is no such thing as a genuine system of milk-vessels, the theory of the circulation of the latex is deprived of its last support.

CHAPTER V.

CONCERNING THE METHOD OF INVESTIGATION.

The successful result of any inquiry depends, to a very great extent, upon the method of investigation which may be adopted; if the method is accurate, the result will be valuable; if, on the other hand, the method be erroneous, the result will prove nothing. The method is accurate when it is adapted to the question which it is wished to determine, and to the object which is under examination. It is necessary, therefore, that the question should be accurately propounded, and that an accurate use should be made of the proper means for solving the question. In order to be able to propound the question properly, it is necessary to know beforehand why the question is put in one form instead of another, and what it is that the answer will determine; in order to be able to make use of the proper means for solving the question, these means, and the effect to be produced by them, must both be known.

Before entering upon investigation, it is therefore necessary to obtain a general acquaintance with the object to be investigated. With regard to philosophical questions which are still matters of controversy, this knowledge only will not be sufficient; in this case it is necessary to be acquainted with the different views which have been taken of the question, and the investigations upon which those views have been founded. Before publishing any philosophical treatise, the writer should not neglect to make himself familiar, as far as possible, with all the recent observations upon the matter in question. By proceeding thus, he will be far less likely to overlook anything of importance, he will obtain more extensive ideas of the subject, his enquiries will be better grounded, he will be able more distinctly to ascertain the value of the opinion which he had

himself formed upon the matter, and will thus arrive at a more certain result. In addition, he will obtain a general historical view of the progress of development of the question at issue.

The great progress which has been made in natural philosophy in this century is owing, in a great degree, to the adoption of the method of induction, which alone is capable of furthering such progress. Although the method of induction leads from individual to general results, that is to say, from the part to the whole, I should, nevertheless, in microscopical investigations, presuppose a superficial general knowledge of the object to be examined. An accurate investigation of the individual parts of the whole will then lead to an accurate acquaintance with the object in all its particulars; in other words, investigation must begin with generalities, must pass from generalities to details, and lead through the details to an accurate acquaintance with the whole.

It will, perhaps, be objected that a superficial acquaintance with an object is unnecessary for the examination of its details. I believe, however, that although in some cases an accurate acquaintance with the whole may be obtained without such superficial acquaintance in the first instance, nevertheless, the inquirer is far more liable to be deceived, and consumes far more time by proceeding without it. In the inquiries connected with the development of plants, I consider it in many cases impossible to arrive at an accurate result without a superficial acquaintance with all the parts of the plant in a perfect state, inasmuch as, without such knowledge, the observer cannot tell what points to direct his attention to, nor what inquiries he should set on foot. I call the knowledge of the entire perfect plant, which is obtained by the naked eye, or by the help of a magnifying-glass, a superficial knowledge, in opposition to the more accurate knowledge which is obtained by a complete examination, within and without, of the individual parts with different magnifying powers. If, in this latter way, the observer has become acquainted with the individual parts, and their relation to one another, he naturally becomes acquainted with the whole plant, and that, not superficially, as in the first instance, but accurately, both within and without.

The course of investigation to be adopted, is, in its fundamental principles, always the same, but it must, as has been observed, be modified in different ways, according to the sort of question which is required to be solved, and the nature of the object to be examined. The investigation of the outward form will require a different mode of proceeding from that which must be adopted in inquiring into the circumstances relating to structure. The inquiries into the development of different portions of plants must be conducted differently from those connected with the development of the cells. It often happens in the course of an investigation that the inquirer is led aside to a collateral question; it not unfrequently happens that the principal question itself becomes essentially changed during the investigation. The collateral questions generally require a particular answer; the principal question must never be lost sight of in answering the collateral ones; particular care must be taken to endeavour to throw light upon the principal question from all possible sources, for which purpose the collateral questions frequently afford opportunity. In this case they must never be neglected. Where, on the contrary, they have no bearing upon the principal question, it is often better in the first instance to pass them by. In carrying out the investigation, care must be taken to pay attention to every point which can in any way facilitate the solution of the principal question; everything must be most accurately weighed, and examined most fully and scrupulously. By this means a safe result will be obtained. The collateral questions which have no bearing upon the principal question, frequently leave materials for future investigations. My own experience leads me to think that it is not advisable to be occupied with many investigations at the same time. One complete investigation sufficiently employs the mind and the time of the observer. The work will not so be well performed if it is always being changed. Inquiries relative to the development of a plant, sometimes form an exception to this rule, since it not unfrequently happens that, in order to follow out the successive developments, it is necessary to examine the same object from week to week, as

I have done in the case of the Yew. In such cases it may be well in the mean time to carry out some other investigation.

From the manifold variety of plants and their different members, it is hardly possible to point out an accurate method of proceeding for all possible cases; the experienced observer will know how to lay out a plan of proceeding suitable to the question proposed, and in accordance with the nature of the object; to the less experienced observer, however, I will give as good advice and assistance as I can. I must here separate the investigation of perfect plants, or of portions of perfect plants, from the inquiries relating to their development, and I prefer to begin with the former as being the most easy. Both divisions of the subject must be treated from two points of view; from the morphological, which relates to their outward form, and the anatomical, which relates to their internal structure. I recommend everybody who is able to draw, to represent on paper, as accurately as possible, all the objects which, in any microscopical inquiries, may appear to him interesting or important; and to add short notes of everything which cannot be accurately represented by the drawing. Too much cannot be done in this way. In matters relating to morphology, simple and accurate outlines are often quite sufficient; in anatomico-physiological inquiries, on the other hand, every individual cell with its contents must be accurately represented. By a series of such drawings, to which in difficult cases mounted objects must be added, a comparison of the different parts of plants, or of the different conditions of development of particular portions of plants, is much facilitated: by this means also, the knowledge of them is advanced, and, in many cases, can only thus be obtained.

Accurate drawings should always be made at the time of everything which appears to be important.

If the observer draws with the camera-lucida, and has some experience in the management of the pencil and brush, and in the use of colour, the loss of time will be compensated tenfold by the value of the drawings. Drawings from memory are in

all cases to be deprecated, inasmuch as they only afford a representation of the observer's ideas, and not of the object itself; these ideas are *subjective*, and therefore liable to be erroneous.

Besides making drawings and preserving objects, it is a good plan to make notes at the time of everything that appears to be important, and even of matters which may not at the time seem to be of much value, inasmuch as during an investigation it is impossible to tell in many cases what influence small matters may have over the result. It is unsafe, especially in extensive investigations, to rely upon the memory; by so doing many things will be forgotten, and many things insufficiently or inaccurately described. Short notes should always be made, at the latest the same evening, of the things which have been observed during the day, and it is useful to add the date of the observation.

It is indispensable also to preserve a memorandum of the magnifying power employed. In difficult cases a note should be made both of the object-glass and eye-glass employed, inasmuch as it is by no means the same thing whether an observation, in other respects similar as regards magnifying power, was made with a strong object-glass and a low eye-glass, or, on the contrary, with a low object-glass and a powerful eye-glass. An observation with a powerful object-glass and a low eye-glass will always carry far more weight. Low magnifying powers are generally sufficient for purely morphological investigations; in these cases it will frequently be necessary to employ incident light; the preparation of the object will here generally be limited to a separation of its parts; the simple microscope and the needle will have to be used more than the knife for the separation of small parts. It will very seldom happen that an observation of the outer form alone will be satisfactory; it will generally be desirable to inquire also into the internal construction of some one part or another; anatomical investigation must therefore be added to the morphological. For anatomical investigations transmitted light is far more generally employed, and the use of the knife will be found of great importance; the needle and the simple microscope will only be of service in

improving thin sections by the removal of unnecessary portions of them. The use of re-agents will throw a light upon the chemical nature of particular parts.

Since, then, morphological and anatomical investigations go hand in hand, I will treat of them both together. I think it is the best plan to begin with the lower orders of plants, as being the simplest products of the vegetable kingdom, and to pass from them to the investigation of the more highly-developed plants. For the same reason, I should advise the beginner to commence his studies with the lower orders of plants; the minuteness of their parts will prove but little hindrance to him when he has gained some dexterity in preparation with the simple microscope. In investigating the more highly-organized plants far greater difficulties will be met with; difficulties will arise which can only be unravelled by an accurate and general knowledge of their construction.

In entering more in detail into the methods of investigation to be employed, the inquiry into the origin of the plant, in other words, the history of its development, must be separated from the investigation of the perfect plant. We will begin with the latter.

On the method of examining the Perfect Plant.—Amongst the Cryptogamic plants, the cellular plants, that is to say, those which have no clearly developed vascular bundles, such as Fungi, Algæ, Lichens, Characeæ, Mosses, and Liverworts, are the most simple. In the first four groups, notwithstanding the great variety of form which exists in their individual parts, no division into stem and leaves can be found; real leaves, that is to say, organs which have a different process of development from that of the stem, appear first in the Mosses and Liverworts. Hardly any preparation is necessary for the examination of the lowest forms of Fungi, such as the flocculent fungi, which is the class to which the different sorts of mould belong; nor is any preparation necessary for the examination of the lowest forms of Algæ, viz., the Confervæ, which consist only of cellular threads. It is sufficient in these cases to disentangle the twisted threads under the simple microscope by the help of a needle, and to clean the plants by rinsing them with water. Particular attention must

be paid to the nature of the cells, both with regard to their walls and their contents; the use of a solution of iodine, and of iodine and sulphuric acid, will often be advisable. The construction of the Characeæ also may be studied tolerably well without any special preparation; they are frequently encrusted with carbonate of lime, which may be removed with very dilute sulphuric acid. In examining the anatomy of the more highly developed Fungi (such as the Pileati and Cupulati), or that of the higher orders of Algæ, such as the Fucaceæ, or that of Lichens, it is necessary to take thin sections from different parts of the plants, and in different but definite directions. Dry Fucaceæ and Lichens may be very well softened by letting them lie for some hours in cold water; the section may be made either with the unassisted hand or between cork. In examining Fungi, fresh specimens only can be used; dry specimens should never be used for examination when it is possible to obtain fresh plants; in inquiries connected with the development of plants fresh specimens are indispensable.

In certain of the Pileate fungi the formation of the spores must be sought for on the under-side of the Pileus, where will be seen the sterigmata, that is to say, stalk-like elongations of the outer extremity of the cells; upon this elongation a spore is formed, which, by the separation of the stalk, becomes free; each spore-cell or basidium generally produces four of these stalked spores. The spores of the higher Algæ are situated partly upon the surface and partly in the hollows of the thallus, and sometimes in peculiar fructifying branches, as in Fucus, where the fruit is developed at the extremities of the thallus, and therefore it must be sought for by making successive transverse sections beginning at the extremity. In lichens the spores are found in peculiar sacs or asci surrounded by paraphyses; the parts of the thallus at which the fructification occurs generally assume the appearance of bowls or cups. As specimens of Lichens, may be mentioned the Borrera Ciliaris and Peltigera Canina, or Peltigera Venosa. Fresh specimens only can be used for examining the development of the spores; a weak solution of iodine renders the asci and paraphyses more or less blue.

The tissue of the higher Fungi, as well as of Lichens, consists of threads formed of cells much entangled with one another; even the gonidial cells of Lichens appear to me to be formed of filamentous tissue, the cells here being only shorter and still more entangled. I have never found the tissue of Fungi to be turned blue by iodine and sulphuric acid.

In the Fucaceæ the form and arrangement of the cells varies considerably according to the species of the plant; since elongated cells are to be found in them it is indispensably necessary that a longitudinal section through the middle of the thallus should be taken in addition to a transverse section. The reagents, such as iodine, the iodized solution of chloride of zinc, and iodine and sulphuric acid, must not be neglected.

In the Floridеæ (a division of the higher Algæ) Nägeli states that he has observed antheridia, that is, organs which, when ripe, discharge moving spiral filaments. Itzigsohn is said to have seen similar organs in Lichens in the spring. Every observer should therefore turn his attention to these organs.

In the Characeæ, as well as in most of the following groups of cryptogamous plants, true antheridia are known to exist. In the more highly developed groups, however, such as the Equisetaceæ and the Ferns, the antheridia are not found in the perfect plant, but at the time of germination. The antheridia are of a much more complex form in the Characeæ than in the rest of the cryptogams; the cells in which the spiral filaments are developed are here strung together like a row of pearls, whilst in the antheridia of all other cryptogams they appear separate; the spores also of the Characeæ differ in their position and construction from the spores of all the other cryptogams. In the cells of the stems of the Characeæ the motion of the juices of the cell may often be well observed; the species Nitella is the best for this purpose; fresh vigorous plants should be taken during warm weather, and examined as soon as possible.

In the Mosses and Liverworts we first meet with a stem and leaves; both parts must here be particularly noticed. The leaves of the Liverwort always consist of a single layer of cells; the mid-rib, which characterizes the leaves of Mosses, is always wanting. In both these plants it will generally be sufficient to

examine the leaves externally, but it is not so with the stem; careful longitudinal and transverse sections must be taken from the stem, either with the unassisted hand (which may be done by a little perseverance), or by placing it between cork. It is, moreover, by no means impossible to make thin transverse sections of the leaves. By treating the stem of Cinclidium stygium, or the leafy stem of Diplolæna Lyellii, in this manner, the first indications of a central vascular bundle will be seen, consisting of elongated narrow cells; in Sphagnum, on the other hand, is to be found a concentric ring, consisting of elongated, thickened, brown cells, surrounded by large perforated cells. In Plagiochila and, as I believe, in all leafy liverworts, as well as in many mosses, the cells of the circumference of the stem are thickened, but every indication of the vascular bundles is wanting. The whole construction of these little plants is far more complicated than that of the before-mentioned groups; this complexity is seen particularly in the construction of the reproductive organs; in these plants we meet with pistillidia, that is, organs in which the young fruit is developed; and we generally find leaves which protect the pistillidia. In Liverworts, the morphology, and the form and arrangement of the leaves with respect to the stem, as well as of the perichætial leaves and perigone with respect to the fruit, can be best studied with the simple microscope, or upon the stage of the simple microscope by the help of a magnifying-glass. The bent needle, or the knife-shaped needle, is of great service here for separating particular portions of the plants. In the ripe fruit, attention must be paid to the construction of its wall, and to its contents. Thin longitudinal and transverse sections made through the half-ripe fruit of a moss afford a beautiful explanation of the construction of the fruit, of the peristome, of the calyptra, &c. The ripe spores must be examined in the same way as pollen; that is to say, in the dry state, and immersed in water, in oil of lemons, and in concentrated sulphuric acid. In examining the elaters of Liverworts, attention must be paid to the nature of their connexion with the fruit, and to the arrangement of the single or double spiral band within the cell. The cell is liable to be overlooked on account of the delicacy of

its walls. Mosses and Liverworts are furnished with antheridia, and the points for the observer to notice are, what position they occupy on the plants; whether they are situated on the same plants as the pistillidia, or on different plants; what is the time of their appearance, what is their form, whether elongated or round, whether their stalks are long or short, and lastly, whether they are provided with a single outer coat, as is the case with most Liverworts, or with a double coat, as in Haplomytrium. If the antheridium is ripe, it generally bursts of itself when placed upon a slide in water; the time in which this takes place varies from five to fifteen minutes. In order to see the spiral filaments properly, it would be best to make use of the strongest object-glass and the lowest eye-glass; the addition of a solution of iodine immediately puts a stop to all motion. The form of the spiral filaments is often best seen after their movements have stopped. Particular attention should be paid to the cilia, which are found in the spiral filaments of Ferns and Equisetaceæ.

In the Rhizocarpeæ (which, according to the recent investigations of Mettenius, certainly belong to the Cryptogamia), as well as in the groups of plants after mentioned, clearly-defined vascular bundles are found, in addition to the leaves and stem. The spores and antheridia appear, in the perfect plant, either separate, or united and enclosed in peculiar protective organs. In Salvinia and Pilularia very good transverse and longitudinal sections of the stem and leaves may be obtained by the help of the cork; the protective organs of the spores and antheridia must be cut through with the unassisted hand. The same rule will apply to the spore, which must be placed upon the finger, and treated with the razor in the same manner as is hereafter recommended for the ovule.

In the Lycopodiaceæ, Equisetaceæ, and Pterideæ, the stem, the leaves with the developed stomata, and the organs of fructification, should be particularly examined. It is important to observe the arrangement of the parts of the vascular bundles in the stem and leaves; the direction of the longitudinal section must therefore be regulated by the arrangement of these parts, which can be ascertained by a transverse section. It is also

very important to trace out accurately the course of the vascular bundles, and particularly the origin of the new vascular bundles, and their connexion with those already existing. In these three groups, the antheridia are never found on the perfect plant; they have been clearly proved to exist during the germination of Equisetaceæ and Pterideæ; they are also to be found, according to Mettenius, in Isoetes and Selaginella (Lycopodiaceæ). The presence of the antheridia at the time of germination may be concluded from the presence, at that period, of pistillidia (or, as I should rather call them, germ-organs); when antheridia are found on the perfect plant, pistillidia are generally found also, as is the case with Mosses and Liverworts; in Ferns and Equisetaceæ the germ-organs, which are analogous to the pistillidia, have been already detected. The so-called spores of the Characeæ are, in my opinion, properly likened to pistillidia, in the interior of which the germ is formed. The nature of the antheridia, and especially their relation to the pistillidia, or germ-organs, has not yet been sufficiently explained in any group in which they have hitherto been detected.

In the Lycopodiaceæ the fruit is found in the axils of the leaves, frequently upon branches specially formed for the purpose; in the Equisetaceæ the fruit is collected in ears on the under-side of certain protecting scales, similar to the anthers of the Coniferæ. The ferns in some instances (such as Pteris Aspidium, &c.), are provided with stalked thecæ, which are collected in small heaps near one another, generally on the under-side of the leaves, and which burst open when ripe; in other cases, such as Botrychium and Osmunda, the spores are developed in sessile leathery capsules, placed on peculiar fruit-leaves. I recommend the use of concentrated sulphuric acid in examining spores in general, and particularly in examining the spores and thecæ of the last-named groups. By using this, the number and nature of the coats of the spores may be observed. Schulz's method of maceration is peculiarly well adapted for obtaining an accurate acquaintance with the cells of stems and leaves.

In phanerogamous plants, the axis (*i.e.*, the stem, branches,

and roots) and the leaves must be separately considered, and examined in a particular manner. The examination of a monocotyledonous stem must be conducted somewhat differently from that of a dicotyledonous stem.

The Examination of the Stem.—In examining the monocotyledonous stem, particular attention must be paid to the arrangement of the parts of the vascular bundles, and to the position of these bundles *inter se;* for which purpose a very thin transverse section must first be made. When, by means of that section, the observer has satisfied himself as to the distribution of the dispersed and definite vascular bundles, and as to the position of the essential parts of the vascular bundles themselves, he must make thin longitudinal sections in different, but definite, directions through the vascular bundle, in order to obtain a clear idea of the nature of its elements. Attention must first be paid to the cambial cells of the vascular bundles, then to the nature of the vessels, and finally to the ligneous cells of each bundle. In monocotyledonous stems it should further be observed whether a kind of bark can be distinguished, as is the case with palms. If this is so, particular attention must be paid to the parenchyma between the vascular bundles, in order to ascertain whether, from the nature of the cells of this parenchyma, it is possible to distinguish a state of tissue intermediate between the bark above mentioned and the proper woody fibre. For an investigation of this nature, fresh specimens are indispensable. This question, as well as all other matters relative to the vascular bundle generally, are, in my opinion, far from settled. In the vascular bundles of Epipogum Gmelini, and Goodyera repens, which, when a transverse section of the stem is made at certain heights appear dispersed in all directions, I can certainly trace a branching, nay more, a regular progression of whole bundles through successive ramifications out of a single central vascular bundle of the root. In some palms which I lately examined (Rhapis flabelliformis) I found the vascular bundles divided underneath the summit of the axis. In the embryo of the seed of the date the vascular bundles of the cotyledon are branched; the place of formation of the vascular bundles lies underneath the plumule.

An accurate investigation of the course of the monocotyledonous vascular bundle is very much to be desired for the sake of science in general. Many methods may be adopted for this purpose. First, if the stem is rotten, the vascular bundles may be laid bare if they are separated by thin-walled parenchyma; where, on the other hand, they are surrounded by wood parenchyma, this tissue must be carefully removed with a sharp-pointed scalpel or penknife, and the course of one or more of the vascular bundles be followed out. Lastly, the increase and change of position of the vascular bundles may be shown to exist by means of transverse sections, made at different heights, proceeding from the root or from the lower part of the plant upwards; and then, by means of corresponding longitudinal sections, the mode of this increase of the vascular bundles must be sought after. In this latter manner I observed the division of the vascular bundles in the Epipogum. It will be a good plan accurately to observe and make notes of the distances of the transverse sections from one another. The epidermis of monocotyledonous stems must also be attended to. In some palms there is to be seen a layer of cork, more or less highly developed, which probably originates beneath the epidermis. The roots of monocotyledonous plants, as far as my observations go, always have a single central vascular bundle, or rather, a crown of vascular bundles, which, in the root of sarsaparilla and in the roots of palms, &c., is separated from the outer layer, which may be called bark, by a row of very thick and generally very narrow cells. In the arrangement of the parts of this central vascular bundle may be seen (sometimes very clearly) the individual vascular bundles which together form the crown of vascular bundles. The root is clothed on the outside with an epiblema, which frequently sends out long hairs. The examination of the root is conducted in the same way as that of the stem.

In the dicotyledonous stem also a transverse section must first be made; this is done by the help of a very sharp razor, either with the unassisted hand, or, if the fragment is small, then between cork in the manner pointed out in page 7. The transverse section must be very thin; the first thing to be

looked to is the arrangement of the parts of the stem from the interior to the exterior, which may be divided into four parts: 1st. The Pith; 2nd. The Wood; 3rd. The Cambium; and 4th. The Bark.

1st. In the case of the Pith, the size and form of it, the nature of the cells, the transition from pith-cells to wood-cells, and, lastly, the contents of the pith-cells must be observed. In some tropical twining plants the pith is not round, but has an angular form.

2nd. As to the Wood. This surrounds the pith, and in it notice must be taken of the arrangement of the medullary rays, that is, those cells which pass in a radiant manner from the pith to the bark; it must be ascertained whether they appear in single rows or in many rows, whether they extend collectively as far as the pith (as is the case in all young plants), or whether some of them being, as it were, secondary rays, are lost in the wood-circle; whether they are numerous and near one another, or fewer in number and at a distance from one another; and lastly, what becomes of them when they reach the bark. The arrangement of the wood-cells must also be observed, that is, whether they are intermixed with vessels, or whether real vessels are wanting, as is the case with the Coniferæ and Cycadeæ. In the Coniferæ particular attention must be paid to the position of the pits, so as to see whether they are only to be met with in the direction of the medullary rays, or whether they are also to be found (which is less frequently the case) in the opposite direction. It must, moreover, be ascertained whether the stem contains turpentine canals, and the position of these canals on the inner side of the annular ring must be noticed. In angiospermous plants the arrangement and size of the vessels and the distribution of the surrounding wood-cells is of importance. In all dicotyledonous stems notice must also be taken of the limits of the annual rings, and it must be seen whether they are strongly or slightly developed, or whether they are wanting altogether, as is the case with most tropical trees.

3rd. The Cambium. In observing this, particular attention must be paid to its connexion with the wood on one side, and

with the bark on the other. The transverse section must be thin and cleanly made, so that the number of rows of cambium-cells, and their nature and contents, may be clearly observed; weak alkaline ley is frequently effectual in removing the granular contents, and rendering the cells more transparent. The contents of these cells must first be tested with a solution of iodine.

4th. The Bark. In examining this, the attention must first be directed to the presence and arrangement of the liber-cells. It must be seen whether they are arranged in bundles or in rows, as is the case with the Cupressini. It must be observed whether the epidermis, which it is certain is never wanting in the young state of a plant, is still to be seen; whether there is any layer of cork, and what is the extent of it; whether turpentine canals or laticiferous vessels are present in the bark; and, lastly, whether the parts of the bark appear to have been deposited with tolerable regularity, and to have been periodically sloughed off, as is the case with the beech; or whether the parenchyma increases in thickness and forms bark without peeling off, as is the case with the oak and the birch.

Besides the above-mentioned transverse sections, longitudinal sections of two different kinds are necessary in examining dicotyledonous stems. The first is a longitudinal section parallel with the medullary rays, and which may be called a radial section. This section must pass from the pith through the wood, the cambium, and the bark; it is only in very thin stems or branches that it is possible to obtain a perfect section of this nature; the difficulty of getting a perfect section is such that it is generally necessary to put up with many different sections, of which one may exhibit the pith and the heart-wood, *i. e.*, the oldest wood surrounding the pith; a second may perhaps show the middle of the wood, and a third the outer part of the wood as well as the cambium and the bark. The same difficulty occurs in making a transverse section of a large stem.

The second kind of longitudinal section which it is necessary to make is one at right angles to the medullary rays, which may be called a tangential section. A section of this nature about the middle of the wood will generally be satisfactory.

In examining the radial section the same course must be pur-

sued as with the transverse section, that is, the attention must be directed separately to the Pith, the Wood, the Cambium, and the Bark.

The Pith.—In this the length and contents of the cells as well as the porous nature of their walls must be noticed.

Secondly, the Wood.—In examining this it must be observed whether the medullary rays are long or short, narrow or wide, whether the pores are large or small, whether any pits are to be seen, and what may be the contents of the cells. Attention must also be paid to the wood-cells and to the presence of a wood-parenchyma which is very beautifully developed in the Leguminosæ. This parenchyma frequently produces starch, which is never present in true wood-cells. The size and position of the pits, and the form and arrangement of their pores, must also be noticed, as well as the existence of a more or less clearly developed spiral in the wood-cells. This latter occurs in the Yew and in the wood-parenchyma of Ulex and Spartium. It must be seen whether the partition-walls of the cells, by the absorption of which vessels are formed, impinge upon one another directly or in an oblique direction; in the former case they will be pierced with a round hole, in the latter the partition-walls will be divided in a scalariform manner, as is the case with Alnus and Thea. These two forms seldom occur together in the same stem. Moreover, the nature of the thickening of the vessels must be considered; *i. e.*, whether they appear to be spiral or scalariform vessels; whether they bear pits; and whether the pits and spirals occur simultaneously, as is the case with the lime. In the Coniferæ inquiry should be made as to the existence of turpentine canals, and also as to the cellular threads pointed out by Hartig, which are isolated, usually narrow cells, with partition-walls impinging directly upon one another, and which contain resin; these latter are found in Thuja, Cupressus, Taxodium, Juniperus, Chamæcyparis, Pinus, and Cedrus, but they appear to be wanting in every instance in which turpentine canals exist in the wood.

Thirdly, the Cambium.—In examining this the form and contents of its cells must be considered, and its gradual transition on the one side into the wood, and on the other into the bark.

Fourthly and Lastly, the Bark.—In examining this, attention must be paid to the parenchyma and its contents, to the shortness or length of the liber-cells (for which purpose the liber of Peruvian bark or of Pinus may be examined), and to the formation of deposits in the liber-cells. The construction of the laticiferous vessels, when present, and the course which these vessels take, as well as the construction of the cork-cells of the tuberous layer, must also be investigated.

The tangential section is especially important for the examination of the wood, and of the arrangement of the medullary rays. It may be ascertained by the help of this section, whether the medullary rays form, in the direction of their length, a single row of cells, or whether, in their middle, they consist of more than one row or of numerous rows of cells, in which case they would appear in the transverse section to be ventricose in the middle and pointed at both ends. This is the case with Laurus sassafras and Hernandia sonora, and more or less with all the Leguminosæ and most dicotyledonous woods. The medullary rays of Ephedra form two or three rows of cells. When the medullary rays are ventricose, the course of the wood-cells is necessarily a tortuous one. In the Coniferæ, the length of the medullary rays must also be observed. The Juniper has medullary rays consisting of from one to five, and the Yew of from two to twenty-four cells. In the Coniferæ, there are sometimes to be found horizontal turpentine canals. They occur in the interior of certain of the medullary rays which are of greater width than the other medullary rays, and are sparingly distributed. These canals are to be found in Pinus sylvestris and Pinus maritima.

The tangential section is important in the case of the Coniferæ for showing the construction of the pits. The lenticular space, and the canaliculus which runs from each of the neighbouring cells into this space, may here be observed. The Yew and the Pinus maritima afford excellent examples.

For preparing the specimens, the rules given in Chap. 3 will apply. With regard to the Coniferæ, it is a good plan to moisten the surface from which the section is to be taken with alcohol instead of water, and it will be advantageous, in almost

all cases, to lay the section in alcohol before making any observations upon it, partly for the purpose of driving out the air, and partly for dissolving the resin. In the case of the Coniferæ, this soaking in alcohol is indispensable. If it is wished to study more minutely the structure of the individual cells of the stem, I recommend Schulz's method of maceration, and the application of a solution of chloride of zinc and iodide of potassium to the cells thus macerated. Very hard woods, such as the woods of tree ferns and palms, may advantageously be laid in water for from twenty-four to forty-eight hours. By so doing, the wood-cells seem to become more tender, and may be cut more easily. The transverse sections of some very hard woods, if made very thin, always roll up. In this case nothing can be done beyond drawing the parts from one another with a needle, and pressing them flat under a tolerably thick covering glass. Thin sections of soft woods often fold together, in which case they must be placed under the simple microscope and spread out by means of a needle.

The same course may be pursued in examining the roots of dicotyledonous plants as has been recommended for the stem. In the true root, *i. e.*, the prolongation of the radicle, the central pith is not wanting; in the lateral roots, on the other hand, it never appears to be present.

In examining fossil woods it is sometimes useful to digest them for several days in a solution of carbonate of soda, and then to wash them clean with water. By this means very good sections may generally be obtained of wood, which, without such treatment, would not be manageable. Very good sections of wood which has been impregnated with carbonate of lime may be obtained by using a watch-spring-saw, and afterwards polishing the sections.

The best plan is to make an even section with the saw, to polish the section upon a fine grind-stone with water, and then to use the saw again. A tolerably thin section (longitudinal or transverse) having been thus obtained, it should be fastened with sealing-wax by its polished side to a cork; the coarse parts of it should then he removed with a file, and the section should lastly be ground completely fine upon a grindstone

under water. The cork with the section should then be laid in alcohol, by which means the section is detached. The section must be cleaned with a camel's-hair brush, and mounted in copal varnish, or Canada balsam. This process may also be adopted in making sections of bone or teeth. With regard to petrified wood, the only plan which can be pursued with advantage is to break off small lamellæ by careful strokes with a steel hammer; the processes of sawing and polishing are generally too tedious for this sort of wood, and seldom lead to a satisfactory result.

The examination of Leaves.—In examining leaves, the first thing that is necessary is to make thin longitudinal and transverse sections through the leaf. If the leaf is not very fleshy the sections are best made by the aid of the cork. In examining the epidermis of the leaves of the species Aloe and Agave, and of all other very fleshy leaves, it is necessary to detach the epidermis, together with some of the subjacent cellular layers, and to place it between cork, since there is no other way in which a sufficiently thin section can be obtained.

The first thing to be done is to examine the epidermis, and to ascertain whether both sides of the leaf have the same sort of epidermis, and whether or not it is furnished with stomata; the construction of the stomata themselves, as well as their mode of arrangement, may be learnt by the help of the transverse section, and by examining the detached epidermis from above. With respect to the stomata, their position and arrangement must be observed, and it must be ascertained whether they are spread over the whole surface of the epidermis or are only to be found upon certain parts of it; whether the arrangement of them is regular or irregular: and whether they are on a level with the epidermis, or raised above it, or sunk below it. The nature of the cuticle is learnt by taking very thin transverse sections and treating them with the iodized solution of chloride of zinc, or concentrated sulphuric acid, by boiling with caustic potash, or by maceration in the manner proposed by Schultz. By proceeding thus, it will be seen that that which most authors call the cuticle, embraces two things; that it consists on the outside of a structureless secretion from

the epidermal cells, and on the inside of the outer layers of the epidermal cells themselves chemically altered. These two parts are generally so closely united that they cannot be separated from one another by concentrated sulphuric acid and maceration; by boiling with caustic potash, however, the individual epidermal cells in the plants Gasteria obliqua, Phormium tenax, and Viscum album, become separated from one another, whilst the secretion from the epidermis is dissolved into a granular matter. A comparison of young and old leaves is here very useful. The hairs which clothe the epidermis, their mode of insertion and construction, must also be observed, and the arrangement of the parenchyma of the leaf, and the distribution of the vascular bundles in the form of nerves, are also of importance. It very seldom happens that the parenchyma of the upper-side of the leaf corresponds in its arrangement with that of the under-side; it often happens that one side has air-holes and the other none. In the plants which are furnished with laticiferous vessels these vessels are generally found to exist in the leaf. The contents of the cells of the parenchyma and of the epidermis also deserve to be noticed. The directions previously given will apply to the examination of the leaf-stalk. Very delicate petals may be advantageously cut between cork. By the aid of the cork I have not unfrequently succeeded in obtaining very thin transverse sections of the leaves of liverworts which consist of only one layer of cells. The cork which is used for delicate petals must be very soft, and the pressure exerted upon the petal must not be too severe.

The examination of the Flower and Fruit.—In examining the flower, the first thing to be observed is the number and position *inter se* of its parts, and afterwards the construction of these parts themselves must be enquired into. For ascertaining the number and position of the parts of the flower, it is best to take moderately thin transverse sections at different heights through an unopened bud. Such a section from the summit of the bud will generally only show the relative positions of the calyx, and the petals, and their situation in the bud. A transverse section made somewhat lower down will, in the case of hermaphrodite flowers, exhibit also the anthers and

their relation to the petals, and frequently also the style or stigma. In plants with superior ovaries this section will also show the relation of the stigma to the surrounding parts of the flower. A transverse section made still lower down will generally be required. In flowers with inferior ovaries it is necessary to make transverse sections at different heights through such ovaries. By means of such transverse sections, which must not be too thin lest the individual parts should fall to pieces, the plan of the flower is seen, and a knowledge of the arrangement of its part is most easily obtained; the different whorls of leaves may thus be clearly observed, and it may be seen how the calyx and the petals are arranged in the bud, what the anthers are like before bursting, whether the sepals and petals and the filaments alternate with one another or not, and moreover, the relation of the divisions of the ovary to the preceding whorl of leaves may also be observed, &c. In transverse sections of this nature, particular care must be taken not to displace any portion of the section by touching it with the needle or any other instrument. In young buds this may easily be avoided by a little care; but buds which are on the point of opening cannot be used for making transverse sections. These sections, and indeed all sorts of sections, are removed from the knife with a fine camel's-hair brush. Sections which are not made quite horizontally through the bud are of no value.

Besides the above-mentioned transverse sections, which are of great importance for an exact analysis of the flower, it is also necessary to make longitudinal sections exactly through the middle of the buds, in the directions shown to be necessary by the transverse section. By means of these longitudinal sections, the insertion of the petals and stamens may most easily be observed; and it may also be ascertained whether they originate from nearly the same point as the sepals, or whether they are borne upon a disc; it may also be seen whether in monopetalous corollas the filaments of the anthers are joined to the corolla, and at what point they separate from it. It may also be observed what is the position of the ovary with respect to the other parts of the flower, whether it is

superior or inferior or intermediate between the two, in what manner the style is united to the ovary, and how the canal of the style is connected with the partitions of the ovary. In many cases these questions can only be decided by observing the development of the ovary and the style. The transverse and longitudinal sections of the bud afford considerable information concerning the nature of the hairs which clothe the blossom. In the Compositæ, a longitudinal section through the whole capitulum must be taken, in addition to the sections through the individual blossoms.

When by the above means a general knowledge of the flower has been obtained, the attention must be directed to the examination of its individual parts.

All the above general directions with respect to the anatomical examination of the leaves will apply to the bract and the calyx. In making an analysis of the flower, its exterior must first be observed, that is to say, its form and colour, and the nature of the hairs with which it is covered; it must then be ascertained whether the tissue is succulent, woody, leathery, or dry, and the changes which take place in the tissue after the period of blossoming must also be noticed.

There is but little to be said with respect to the petals. By the aid of thin transverse and longitudinal sections, made between cork, the construction of the petals and of their epidermis may be ascertained. By examining the whole surface of the petals with a low magnifying power, or with incident light, the distribution of its vascular bundles is made clear. It is these bundles which frequently produce the delicate lines upon the petals. The fluid contents of the cells, which in the petals are frequently of such beautiful colours, must be particularly noticed. The form of the petals, their colour, and the nature of their exterior, are important in analysing the flower. In dealing with the stamens, the anthers must be particularly attended to; they must be examined out of the bud before the latter has opened, and also shortly before and after dehiscence; in the latter case a transverse section is seldom practicable. The anthers, whilst in the bud, will generally be found to be quadrilocular. The cellular mass which divides the two loculi

of each side is afterwards either wholly or partly absorbed, so that, at the time of the flower opening, the anther appears to be only bilocular. Both unilocular and bilocular anthers are, however, to be met with; the anther of Callytris is unilocular, and probably, also, the anthers of all Coniferæ and Cycadeæ; bilocular anthers are to be found amongst the Amaranthaceæ, in Gomphrena decumbens, and Alternanthera diffusa. Albersia and Celosia, however, have normally quadrilocular anthers. The anther of Meryolix serrulata develops its pollen in detached groups from mother-cells; it is not until some time afterwards that the parenchyma which divided these groups disappears; the anther opens, as in the rest of the Onagraceæ, with two longitudinal fissures. In Viscum, also, the mother-cells appear in groups, separated from one another by parenchyma. Since these circumstances cannot always be predicated with certainty, it is indispensable for an accurate analysis of a flower, that a transverse section be taken through the anther whilst in the bud. The connective of the anther, which is always characterized by its vascular bundle, must also be examined, as well as the loculi and their walls, and particular attention must be paid to certain cells which are generally to be found in the walls of anthers, and which contain a delicate spiral band.

In considering the anthers morphologically, we must observe the nature of their attachment to the filament, their mode of dehiscence, and the form of their valves. It must be seen whether they are provided with elongated processes at either end (which may be well observed in the Compositæ), and whether the loculi are fully developed on both sides of the connective, or whether only one loculus produces pollen, as is the case in the Salvia. The form, also, of the filaments must be accurately observed, so as to ascertain whether they are short or long, straight or bent, simple or furnished with appendicular processes. The latter is the case with Asclepias and Borago. The insertion of the filaments must also be looked to, and it must be seen whether they appear to be united to one another, and what is the nature of the connexion, if any.

The contents of the anthers, *i. e.*, the ripe pollen, must be carefully considered. It is examined dry, and also in water, in

oil of lemons, and in concentrated sulphuric acid; in some cases it will also be judicious to treat it with the iodized solution of chloride of zinc. In examining the pollen particular attention must be paid to the number of its coatings, and to the openings destined for the egress of the pollen-tubes. The number and disposition of these openings must be considered, as well as the questions whether they lie in depressions of the outer coating of the pollen (which can frequently only be ascertained by treating the pollen under water), or whether they are provided with opercula, as is the case with the Stellaria. The construction of the outer coating, which frequently assumes the most delicate forms (the beauty of which may be seen in the Cichoraceæ, in Stellaria, the Cucurbitaceæ, and Amaranthaceæ) merits attention. In treating the pollen-masses of the Asclepiadeæ, thin sections should be taken between cork. The leathery outer layer, which turns red upon the application of concentrated sulphuric acid, will then be seen to be a secretion from the pollen-cells. In orchideous plants, the connexion of the pollen-masses with the caudiculus and the retinaculum must be observed; each pollen-mass must first be examined separately, and afterwards the individual grains of pollen, which latter must be submitted to different tests. It will frequently be well worth while to observe the egress of the pollen-tube, especially when the pollen appears to have only one coat; it is generally easy to obtain pollen-tubes by applying the pollen to the stigma; they generally appear in numbers after a time varying from one to eight days. The moisture which is secreted from the stigma of the flowers of Hoya carnosa is well adapted for the formation of pollen-tubes; if the pollen of other plants is brought to their style very beautiful pollen-tubes are generally obtained. The use of eau sucré is seldom attended with any success.

We will now consider the style and stigma. These may exist singly or in greater numbers. Thin longitudinal sections are generally sufficient. In the stigma attention must first be paid to the tissue which secretes a fluid matter, and which is generally covered with papillæ. In the style the most important points to be attended to are, the course of the canal and the nature of

its conducting tissue. A thin transverse section through the style is often useful; by means of it the distribution of the vascular bundles may be ascertained.

In examining the ovary it is necessary to make very thin transverse sections at different heights. If the ovary appears to be multilocular it will sometimes be necessary to have recourse to the needle and the simple microscope to determine whether such is really the case. Very many ovaries which are stated in books to be multilocular with a central placenta, are, in reality, unilocular ovaries with many parietal placentæ lying close to one another. I may mention as examples the genera Œnothera, Clarkia, Escallonia, as well as the Cucurbitaceæ, &c. In the three Onagraceæ just mentioned there are four parietal placentæ which, in a transverse section, project like a border into the hollow of the ovary. They spread out at their extremities on both sides, bear an ovule on each side, and lie close to one another. Between these four placentæ, which are in contact with one another, there is formed an open space, which is, to a certain extent, a prolongation of the canal of the style; the four placentæ grow together at the lower part of the ovary. In examining the transverse sections of the ovary, the attention must also be directed to the arrangement of the placentæ, and to the distribution of the ovules upon the latter. The distribution of the vascular bundles in the ovary and in the placentæ should also be observed, as well as the hairs which clothe the ovary. The longitudinal section through the middle of the ovary is regulated, partly by the arrangement of the placentæ, and partly by the position of the style and stigma. It will frequently be necessary to make longitudinal sections in different directions through the middle of the ovary, and, if possible, also through the middle of the style, and through part of the stigma. It will more frequently happen that from the impossibility of making such longitudinal sections the stigma and style must be examined separately. In examining longitudinal sections of the ovary the attention must again be directed to the placentæ and ovules, to the position of the latter, to the connexion of the canal of the style with the hollow of the ovary, to the distribution of the vascular bundles which pass

from the peduncle into the ovary, and to the subsequent ramifications of these bundles in the other parts of the flower.

The most important part of the ovary is the ovule, the condition of which at the period of blossoming must be considered. Three things must be attended to in considering the ovule. 1st. The existence and the number of the coats of the ovule; 2ndly. The position of the ovule, and especially the situation of the micropyle with regard to the hilum; 3rdly. The situation of the embryo sac, and its relation to the nucleus.

These questions can seldom be solved by examining the whole ovule; in the Orchideæ, in Monotropa, and in certain species of Pyrola, the ovules of which are very small and transparent, and whose delicate nature precludes the possibility of preparing them in any way, it is possible, by accurate adjustment, to examine the ovules entire. In most cases, however, thin longitudinal sections must be made exactly through the middle of the ovule; in particular cases, such as the Œnothera, the best way of doing this is to make thin longitudinal sections through the ovary itself; amongst the many ovules which will be thus cut through, some will be found here and there to have been accurately divided; these must be extracted under the simple microscope. In other plants, such as the Iris and Cucurbita, a transverse section is more advantageous. In almost all other cases it will be necessary to detach the ovule itself, to place it upon the forefinger, and to make two cuts through it with a very sharp razor, so as to obtain a thin longitudinal lamella forming exactly the middle of the ovule. The best way of doing this is to slice off one side of the ovule, to turn the ovule round with a fine camel's-hair brush, and then to slice off the other side of the ovule. The section thus obtained must be placed under the microscope; if it is only a moderately good one it may frequently be improved by a third or fourth cut made in the same manner. The correct position of the ovule upon the finger must first be ascertained by the help of a lens. The ovules of all the Personatæ, of the Labiatæ, of the Borragineæ, of the Coniferæ, &c., require to be treated in this manner. In particular cases the existence of the coats of the ovule cannot be clearly ascertained even with the most successful sections—

this is particularly the case where the nucleus is very slightly developed and very soon displaced by the embryo sac. In this case, unless the nature of the development is known, it must be doubtful whether a naked nucleus or a single highly-developed integument is present. As an instance of this I may mention Asclepias Syriaca, the development of which will be fully considered hereafter.

With respect to the position of the ovule, three principal types may be mentioned. 1st. The orthotropal ovule, where the micropyle lies in a direct line over the hilum, as in Hydrocharis, Taxus, and Juglans; 2ndly. The anatropal ovule, where the micropyle lies near the hilum, and where the raphe or vascular bundle of the funiculus runs along one side of the ovule, as in Cucurbitaceæ, Irideæ, Liliaceæ, Impatiens, Viola, and Orchideæ. In this, as well as in the first-mentioned kind of ovule, the chalaza, that is, the place where the vascular bundle of the funiculus terminates, lies opposite to the micropyle, and the nucleus and embryo sac are not bent. The third species of ovule is the campylotropous ovule. In this case the development of all the parts has taken place only on one side. The micropyle lies near the hilum, the raphe is very short, and the embryo sac is bent. There are numberless intermediate forms and modifications of these types to which, in some cases, special names have been given; such names, however, are insufficient to characterise them. Careful drawings of different kinds of ovules will bring out their peculiar forms far better than the most ample written descriptions.

In considering the embryo sac it is especially important to attend to its relation to the nucleus. In the Orchideæ and Personatæ the nucleus is at an early period displaced by the embryo sac. In the Rhinanthaceæ, Orobancheæ, Acanthaceæ, and Labiatæ, the embryo sac frequently produces sac-shaped elongations which absorb the parenchyma of the single integument, break through it, and often protrude into the hollow of the ovary. The only way of obtaining a clear view of this very interesting condition of the embryo sac is by means of a very thin longitudinal section carried through the middle of the ovule. Attention must also be paid to the presence of a real

endosperm at the time of flowering. This may be observed in the Personatæ, Hallorageæ, and Hippurideæ. It is also important that the observer should satisfy himself as to the existence of peculiar cells at the apex or at both extremities of the embryo sac.

There are many other collateral organs of the flower, such as the imperfect stamens, the nectaries, the disc, &c., to which it is not necessary more particularly to refer. Whoever carefully follows the directions here given, cannot possibly overlook any such organ if it be present.

The directions given for the examination of the ovary will apply to the examination of the ripe fruit. Particular attention must here be paid, in an anatomical point of view, to the changes in the formation of the tissue, to the absorptions which are found to take place, &c. Morphologically the form and the nature of the dehiscence of the fruit will be of importance, and it will be necessary also to observe the changes of the other parts of the flower, and to ascertain whether they fall off soon after the period of flowering or whether they remain behind, and what effect they have on the form of the fruit or upon its condition.

The ripe seed is examined in the same way as the ovule, and in order to obtain a correct idea of its morphology, we have to consider its form and the nature of its outer surface. By means of thin transverse and longitudinal sections, the observer will be convinced of the transformation of the single or double integument into the testa of the seed, and will satisfy himself of the presence or absence of the pre-existing nucleus, the tissue of which, when it exists in the fruit, is called the perisperm, as in the Nymphæaceæ. By means of these sections he also ascertains the presence or absence of the albumen or endosperm, a kind of parenchyma which is formed in the interior of the embryo sac; and lastly, concerning the nature of the cells of the embryo itself.

In making these investigations, the contents of the cells must be tested with iodine, and the iodized solution of chloride of zinc. In examining the embryo itself and its position in the ripe seed, it is often advantageous to divide it into two equal

parts, and to take a moderately thin transverse section of it. It is a good plan to soften hard seeds by soaking them in water for twenty-four hours. It will often be advantageous to detach the whole embryo, and to treat it separately from the seed. In difficult cases it must be examined on all sides with incident light under a low magnifying power, and must be illuminated in many different ways. The embryo of dicotyledonous plants will seldom be difficult to examine. The parts to be distinguished are the axis and the cotyledons. The axis is that part of the embryo which terminates in the direction of the micropyle, in the form of a little root, and the other end of which forms the terminal bud or plumule. The cotyledons proceed from this axis. The Coniferæ, the Lime, and some other plants have more than two cotyledons; the Cuscutaceæ, Monotropa, and, amongst monocotyledons, the Orchideæ, have no cotyledons. The plumule is highly developed in some plants. In the Tropæolum, for instance, two complete leaves are to be found. In other plants, on the contrary, such as Pedicularis, Impatiens, and Hippuris, it is only to be seen in the form of a slight protuberance between the cotyledons. The embryo of monocotyledons presents greater difficulties—difficulties, in fact, which frequently can only be got over by attending to the development of the embryo. Accurate longitudinal sections are here of great importance; they exhibit, in the Gramineæ, the development of the lateral roots, and also the sheath from which the first leaves of the embryo afterwards escape, which sheath has two vascular bundles. Much remains to be done towards elucidating the monocotyledonous embryo.

The form and position of the embryo, and the presence or absence of albumen are of importance in systematic botany.

The motion of the juices of the cell must not be passed over in silence. It is not to be seen in all plants, although it may be conjectured that it is present in all living vegetable cells. It may be seen in the simplest form in the hairs of the roots of Hydrocharis morsus ranæ, for which purpose a perfectly fresh plant must be taken on a warm summer's day; those hairs on the roots which hang down in a flaccid manner will not exhibit the motion, but it can generally be found in those

which stand out horizontally from a long thin root. A piece of one of such roots must be brought under the microscope and placed under a covering glass, and one particular hair must be watched continuously and attentively. It is seldom necessary to wait long; the motion is frequently interrupted just at first, but generally begins afresh in a few minutes. The stream flows along the walls of the cell, and is clearly seen to bend back again at the apex of the hair. Thin sections of the young leaf of the Hydrocharis, and of the leaves of Stratiotes aloides and Vallisneria spiralis, exhibit the same motion. In Vallisneria large granules of Chlorophyll are carried along by the stream. The motion in the hairs of the stamens of Tradescantia is more complicated. In these hairs different currents may be distinguished: the larger currents traversing the walls, and the smaller currents passing from the cytoblast to the walls; the direction of the latter frequently changes; they cease, and new ones form themselves. The hairs of the young ovaries of Ænothera and Clarkia exhibit similar movements. Warm, clear days and perfectly fresh plants are necessary for observing these movements. The movement of the contents in the parenchymatous cells, as in the Snowberry and in the youngest cells of the endosperm of Pedicularis, &c., is much less frequently seen; it depends upon a peculiar condition of the cells which may, by good fortune, be sometimes met with. I have observed, on two occasions only, but then in the greatest perfection, a very complicated movement in the prolongation of the embryo-sac of Pedicularis sylvatica. Any person who has carefully observed these appearances, if it be only twice in his life, will easily be convinced that they cannot be explained upon the supposition of any vascular system in the interior of the cells, but that the motion proceeds from a fluid, which is separated from, and does not mix with, the rest of the fluid contents of the cell. By applying iodine, or iodine and sulphuric acid, the fluid is turned yellow, and the motion then ceases.

On the method of investigating the developement of Plants.—In tracing the developement of a plant, it is necessary, if the inquiry is to be of any scientific value, to go back to the primary origin of the plant, or of the part under investigation.

In tracing the developement of the embryo, therefore, it is necessary to show with certainty the origin of its first cell; the developement of the flower must be traced from the appearance of the floral axis, as a simple round cellular little body in the axil of the bract. In conducting the investigation, care must be taken not to overlook any matter of importance; where the investigation is complete, *i. e.* where the developement is followed out in all its successive steps, great service is rendered to science; and it is by so doing alone that clear ideas upon the subject can be formed. In questions relating to developement fresh plants only must be used.

It would not only be tedious, but almost impossible, from their variety, to point out the method of tracing the developement in the individual groups of cryptogamous plants. I will only shortly mention what I consider, at present, to be most requisite for the advancement of science in this respect; and the first thing to be mentioned is *inquiry as to the germination of cryptogamous plants in general.* There are but very few cases in which I can, from my own experience, give directions as to how this is to be done; my attention in this respect having been directed only to ferns and liverworts. The method to be adopted for producing germination, and for investigating it when produced, must vary according to the peculiarities of the plant. I may mention, as a point of great importance, the developement of the reproductive organs, especially the origin of the pistillidium and germ-organs of the higher Cryptogamia, and their hitherto unascertained relation to the antheridia. The formation of the fruit within the pistillidium or of the young plant within the germ-organ, the developement of the spores within the sporangia, the existence of gemmæ, their origin and developement, and lastly, the developement of the true antheridia, and of their phytozoary cells, must also be considered.

Besides the above-mentioned inquiries, many very interesting questions might be suggested, depending upon the peculiarity of the particular group or even genus. For instance, in the higher Algæ, inquiry might be made into the nature of their growth, and especially as to the mode of thickening of their perennial stems, proceeding from the organs of adhesion.

In the fungi, the effect of chemical agents upon the cellular membrane, in its old and young state, might be observed. In the leafy liverworts, an examination into the mode of developement of the perigone, as to which few accurate observations have been made, would be desirable; and with respect to all the Cryptogamia which have stems and leaves, an investigation of the developement of those organs would be very valuable. In order to bring about germination in ferns, the best plan is to take a large fragment of a frond with ripe spores upon it, to place this fragment upon moist garden-mould in a flat earthenware vessel, and to cover it with a glass; the mould must be kept sufficiently moist, and the vessel placed in a tolerably warm shady place. After a time, varying from a fortnight to five weeks, the spores generally begin to germinate; the first indication of which is a green parenchymatous expansion. Pteris serrulata germinates very easily. Some spores should then be taken up and rinsed with water. The antheridia in the younger specimens are very beautiful. When the germ has assumed a leaf-like form, transverse sections must be made between cork: this is important when it is wished to observe the germ-organ and its developement; the germ-organ is closed at first, and afterwards opens. Accurate observations as to the origin of the primary cell, the foundation of the young plant, within this germ-organ, would be of the highest importance to science. In examining the spiral filaments, it is important to observe their developement, their mode of escape from the antheridia and from the mucilaginous cells, the number of their coils, the manner in which they are covered with cilia, the bladder-like expansion which, in some cases, exists at one extremity, the nature of their movements, and the manner in which they are affected by chemical agents.

The spores of liverworts germinate most easily in white moist sand under a glass. The genus Pellia germinates in a few days. Those spores which have two coatings require a somewhat longer time. The developement of the pistillidia in mosses and liverworts may best be seen by taking a thin longitudinal section through the middle of the young stem. They are found, like the germ-organ of ferns, to be always closed

in the first instance, and afterwards they open at their apex. In order to follow out the developement of the spores, thin longitudinal and transverse sections must be taken from time to time through the situs of the spores, from the earliest stage until the spores are ripe. The use of re-agents will here be essential. In order to trace out the origin of gemmæ, it is necessary to observe the transformation of certain cells of the mother plant, and their subsequent developement into gemmæ, which must be done, either by means of longitudinal and transverse sections, or by carefully detaching the particular parts to be observed. In Blasia the gemmæ remain for some time united to the mother-plant by a many-jointed cellular stalk. In the liverworts the pistillidia always make their appearance before the perigone or cup; the formation of the latter seems not to take place until the rudiments of the fruit have been formed in the interior of the pistillidium. The perigone is not formed of leaves grown together; it originates in the form of an annular swelling around the pistillidium, as may be seen in Liochlæna lanceolata and Frullania dilatata.

The developement of the stems and leaves of cryptogams, as well as of their vascular bundles, must be investigated in the same manner as in the case of phanerogamous plants.

On the investigation of the developement of the Stem and Leaves, and of the Vascular Bundles contained in them.—In tracing the developement of the stem and leaves, two modes of proceeding may be adopted. The first is to examine the plant at the time of, and subsequently to, germination; the other is to follow out the developement of the bud, and of the young branch. In order to arrive at a satisfactory result, both methods should be pursued. In both it is necessary, in the first instance, to take very thin longitudinal sections directly through the middle of the apex of the stem. If a section is thus made, the apex, whether it originate from a germ or a bud, will be found to be a small closed protuberance of a more or less conical shape, clothed with an epithelium, and underneath this protuberance will be found a tissue, consisting of small cells quite filled with a granular substance which turns bright yellow on the application of iodine. This tissue loses itself lower down in the

different tissues of the stem. In dicotyledonous plants the transition of the former into pith, wood, cambium, and bark, may be seen; and in cryptogamous plants into parenchyma and vascular bundles. If a very careful longitudinal section is made through the apex of a young branch, the age of the cells may be accurately investigated; the deeper they lie the more fully developed they will be found to be, both in length and breadth, and in the degree of their thickening; they are younger and less developed in proportion as they are nearer to the apex. If a section of this nature is treated with iodine and sulphuric acid, the lower parts of it immediately become blue; towards the apex this change of colour takes place quite gradually, and passes through the most various shades of yellow, and through red and violet, to blue. The conical end of the stem frequently does not turn blue for many hours.

Beneath this conical protuberance, which may be called the terminal bud, or Punctum vegetationis, and on both sides of it, if the section be well made, are to be seen other small protuberances, which are covered with the same epithelium as the Punctum vegetationis, and which consist of cells of the same nature as those forming the tissue of the Punctum vegetationis. These small protuberances appear to be more developed in proportion as they are situated lower down upon the stem; they may easily be seen to be the rudiments of leaves. If the section is sufficiently well made, which, however, is seldom the case, it may be seen that the cells at the apices of these protuberances are larger and more developed than those at their bases; iodine and sulphuric acid produce, instantaneously, a blue colour in the cells at the apex, whilst those which lie lower down, and especially the cells at the base of the leaf, comport themselves in precisely the same manner as those at the apex of the stem, showing thereby that their age is less. This circumstance, which is observed in the stems of both phanerogamous and cryptogamous plants, and which may be well seen in sphagnum and the leafy liverworts, shows that there is an essential difference between the growth of stems and leaves. The stem or axis grows at its apex, the leaf at its base; the apex of the axis is the youngest, of the leaf the oldest, portion.

If a thin transverse section is now taken close under the Punctum vegetationis, there will be found, in dicotyledonous plants, a number of dispersed vascular bundles, the ligneous cells of which are turned towards the pith, and the cambium towards the bark; these vascular bundles are separated by a mass of parenchyma, often of great width, which unites the pith and the parenchyma of the bark. In a very young state of the plant, the wood-cells and vessels are scarcely distinguishable from the cambium, and the liber-cells are generally not yet present. At a subsequent period the different parts are more clearly defined; the liber-cells appear on the outside of the cambium, the vessels become extended, and the parenchyma, which at first separated the vessels from one another, becomes reduced to a narrow remnant constituting the medullary rays. A closed ring of wood is formed, increasing in circumference yearly by additions from the cambium, which on its inner side forms new wood, and on its outer side new bark. In cryptogamous and monocotyledonous plants, where the cambium, from its position, is incapable of forming wood and bark in the manner just mentioned, the stem does not increase in thickness, but only in length. In order to trace the formation of the young wood, it is necessary, both in spring and in summer, to make transverse and longitudinal sections in two different directions; the sections must be extremely thin, and the cambium especially must be cut through quite smoothly. It is advisable to place these sections for a few minutes in a weak alkaline ley. By so doing, the cambium cells frequently become more transparent. In the young wood-cells of Pinus abies a spiral band may be clearly seen, as well as the gradual formation of the pitted vessels.

Very thin longitudinal sections taken from the apex of a young twig, such as those which have been recommended for the examination of the stem and leaves, afford also sufficient information as to the origin of the vascular bundles. It will be seen how all the parts, the cambium, the wood, the vessels, and the liber-cells, originate underneath the Punctum vegetationis; and in going downwards from this point it is possible, by means of very careful sections, to follow out the developement

of these parts, and to perceive, especially in the vessels, the gradual formation of their peculiar thickening layers. It will be seen further, how the increase in number of the cells takes place principally, perhaps solely, in the tissue underneath the apex of the stem, and in the cambium of dicotyledonous plants; and how, on the other hand, the growth of the parts further removed from the Punctum vegetationis depends chiefly upon an increase of size and elongation of the cells. *Cell-multiplication* and *cell-extension* are essentially different. This must always be borne in mind in questions relating to developement.

At the places where young leaves originate, there is to be seen in dicotyledonous plants, on the outside of the vascular bundle, the origin of a branch vascular bundle; this branch loses itself in the parenchyma of the young leaf, and increases in growth, like the leaf, from the base outwards. I have seen the same thing in Epipogum and Goodyera, which are monocotyledonous plants. The vascular bundle of a leaf which is not fully formed is, like the leaf itself, more fully developed at its apex than at its base, at which latter point it progresses in growth by the multiplication of its cells.

The circumstances connected with the formation of leaves appear to be dependent upon the position of the cambium in the stem, at least in the case of dicotyledonous plants. The increase of the leaf by the multiplication of its cells takes place in the vicinity of, and in fact is produced by, the cambium. The leaf, therefore, is distinguished from the axis of the plant in this respect, that the cells by which its growth is increased lie at the base of the leaf, whilst in the axis these cells lie at the apex. The same difference of growth also exists in monocotyledonous plants and in the higher Cryptogams, in which there is no concentric cambium, and the stems of which, on this account, do not increase in thickness, and can form no new leaves. The formation of leaves in these latter plants is limited solely to the upper part of the stem, which lies under the Punctum vegetationis. If thin longitudinal sections are made at this point, it may be easily seen that the layer of growing cells extends downwards from the Punctum vegeta-

tionis, in a campanulate form, around the central part of the stem, until, at a certain depth, it disappears altogether.

The vascular bundles of monocotyledons grow only at their apex, those of dicotyledons both at their apex and in their circumference, the latter having the growing cellular tissue at both places. The vascular bundle of a monocotyledon appears, therefore, to be branched only at its apex, whilst the dicotyledonous vascular bundle sends out side-branches into the new buds and roots. The vascular bundles of all plants, when present, generally appear to have a certain connexion with one another, which connexion depends upon the manner in which they originate. It is, therefore, incorrect to suppose that the stem is formed out of leaves grown together; the nature of its developement shows directly the contrary. In the dicotyledonous embryo the simple central portion—that is, the axis—appears first, the two cotyledons proceed from it on either side, and between the cotyledons lies the plumule, which answers to the Punctum vegetationis of the apex of the stem. The first two leaves, therefore, are formed out of the stem by the division of it, and not the stem by the growing together of two leaves. The further progress of developement corroborates this. At the points where new leaves originate, a side branch of the vascular bundle of the stem is shortly after formed, and above this side-branch there is a small cellular protuberance, which, together with its vascular bundle, forms, by developement, the leaf. The leaf, therefore, receives its vascular bundles from the stem, but a new vascular bundle never originates in the leaf to unite with the vascular bundles of the stem. The same rule holds with respect to the origin of new buds in the axils of the leaves. The first rudiments of new buds, as well as of side-roots, always originate in the vascular bundle of the stem. The only places where the formation of much new substance for the buds and leaves takes place, are, the apex of the axis and the cambium of the vascular bundles of the stem. I have dwelt longer upon this part of the subject, because the point, which is one of much importance, has by no means received the attention it deserves, and but few well-grounded observa-

tions have been made upon the subject. It is of the greatest importance that very accurate sections alone should be used; oblique sections have been the cause of much error. The best way of guarding against this error is, to make the finest possible longitudinal sections through the apex of the stem, to place them together under the microscope, and to select those sections which appear to be cut quite perpendicularly through the stem, as well as exactly through its middle. When such a section is properly made, the Punctum vegetationis always appears in the form of a small cone; if this is not the case the section is either not cut perpendicularly through the stem, or not exactly through its middle. It is, moreover, indispensable to distinguish accurately between the increase in number of cells and their increase in size.

On the method of examining the developement of Flowers.—In examining the developement of the flowers much greater difficulties will be met with. From the minuteness of the object it is impossible always to regulate accurately the direction in which the sections are made, and on this account it is often necessary to select out of many sections those which happen to have been well prepared, and some experience is necessary to be able to distinguish the good sections from the bad. Still greater difficulties arise in the case of irregular flowers. The growth of the different whorls of leaves does not always progress simultaneously; the petals, although they are always formed before the stamens, frequently lag behind the stamens in their subsequent developement, and on that account are sometimes liable to be overlooked. The difficulties above mentioned are sometimes further increased by the circumstance (which occurs in many flowers) of the whorls being at first separate, and subsequently becoming united. Every beginner, therefore, should be advised, before commencing the study of the developement of irregular flowers, to make himself fully acquainted with the developement of regular flowers. The flowers of Ænothera, Clarkia, and Epilobium are well adapted for this purpose. In order to render the investigation more easy, it is necessary to select plants which have a spiked or panicled inflorescence, and moreover plants which have but few

hairs. The longitudinal section through the middle of such a spike exhibits simultaneously in the axils of the bracts, different stages of the developement of the flower. Moreover, when the flowers have but few hairs, the examination of them is much less liable to error, since air is often collected between the hairs, and this must first be removed with alcohol, the use of which, in such young specimens, is often not advisable. In examining the developement of the flowers two modes of proceeding may be adopted. First, the parts of the flower in their successive stages may be prepared separately by the aid of the simple microscope; and, secondly, very delicate longitudinal and transverse sections, in certain definite directions, may be made through the whole of the flower. The second mode of proceeding is decidedly preferable; it is more rapid and more certain in its results; it affords a far more accurate insight into the internal condition of the flower and its parts; and lastly, after a little practice, it is far more convenient and easily managed. In preparing the separate parts, there is no security, notwithstanding the greatest dexterity in the use of the needle, against their being injured; and finally, the observations are rendered difficult by the fact that the parts of the flower must be viewed as *bodies*, by varying the adjustment of the microscope, and cannot, as in the case of the sections, be examined as *surfaces*. In many cases, as for instance in examining the developement of the flowers of grasses, both methods should be used.

In selecting specimens for examination, the youngest flowering branches should be taken. The longitudinal sections should be made with the unassisted hand. The section must be sufficiently thin to exhibit accurately the middle lamella of the flowering branch; and in examining it, the terminal bud and the bracts beneath it must be observed. The first rudiments of the flower will be seen within the bracts in the form of a round cellular little body, precisely similar to the first rudiments of a leaf-bud; this cellular little body is the proper axis of the flower. The sepals will be seen in the form of little round warts, situated in the axils of the bracts, and in a circle round the cellular little body just mentioned. In cases where the section bisects the floral rudiments the apex of the

floral axis is seen between the rudiments of the calyx in the form of a round protuberance, as a plumule or Punctum vegetationis. In such a section the rudiments of the different whorls will be seen in succession one under the other.

Having gained some information by means of longitudinal sections, the observer must then prepare thin transverse sections, proceeding from the apex of the flower. It is often advisable to make these transverse sections in a direction somewhat oblique to the principal axis, inasmuch as the position of the floral rudiments with respect to that axis (*i. e.* the common flowering stem) is generally somewhat inclined. This rule is particularly applicable to those flowers which are situated low down on the stem. It is important, for the purposes of investigation, that the transverse sections should be cleanly made, and that they should be exactly at right angles with the longitudinal axis of the floral rudiments. It is necessary, therefore, out of the number of floral rudiments through which one such section generally passes, to select those which appear to have been divided in the right direction. It is often necessary to make many sections before it is possible to obtain perfect specimens of the different stages of developement. It is particularly necessary to follow out the developement continuously step by step, and therefore it is a good plan to make an accurate sketch of the outlines of all successful sections, both longitudinal and transverse. If such longitudinal and transverse sections, taken from different plants in similar stages of developement, are compared with one another, they cannot fail to lead to a right understanding of the subject. The simple microscope will be found to be indispensable for improving longitudinal sections by the removal of superfluous parts, as well as for detaching, for preservation, particular portions of transverse sections of the entire floral rudiments. Repeated use of the razor is often necessary for rendering longitudinal sections more complete.

In examining the developement of a flower by the aid of a transverse section, the following points must be particularly attended to :—

1. The succession of the floral whorls, and their number.

2. The position of the parts of one whorl with respect to those of the preceding one. If these parts do not alternate with one another, it may be assumed that there is a suppression of an intermediate whorl, and in that case the rudiments of the suppressed whorl must be sought after. It is seldom, however, that this search is successful.

3. The number of the parts of each whorl, and the manner in which they harmonize with one another. When one whorl consists of fewer parts than the preceding, a suppression of some organ may generally be ascertained by observing the position of the parts of the whorls *inter se*: in these cases, the rudiments of the suppressed organs must be sought after, and they will occasionally be found in their right place in the form of inconspicuous excrescences. When, on the other hand, it happens (which, however, is seldom the case) that a whorl has more parts than the preceding one, the first thing to be inquired into is, whether the preceding whorl is complete, or whether the additional parts in question really belong to the whorl in which they are found.

4. The growing together, or adhesion, of the originally distinct parts of one or other of the whorls. This can only be observed by comparing good transverse sections taken at different periods of the developement.

5. The construction of the anthers—as to whether, up to a certain period, they are bilocular or multi-locular.

6. The parts forming the ovary. The superior ovary may be formed either out of the axis or out of leaves. The ovary, which is formed from leaves, may originate from one leaf or many leaves; the number of these parts seldom stands in any definite relation to the parts of the preceding whorl. In very many cases it will be a matter of doubt whether the ovary is formed from the axis or from leaves; the only way of deciding this question is by making very exact observations as to the manner in which the cells of the ovary increase in number, so as to ascertain whether this increase takes place at the apex or at the base. The inferior ovary is always formed from the axis.

7. The origin of the placentæ and ovules, and the nucleus, coatings, and embryo-sacs of the latter. This investigation is

of great importance; it shows whether the dissepiments of the ovary are true or false. In Ænothera, Clarkia, Epilobium, in the Cucurbitaceæ, in Pyrola, Monotropa, &c., they are false; the dissepiments in these plants are only apparent, and are, in fact, formed out of the parietal placentæ.

Transverse sections of the stigma and style will seldom afford satisfactory information.

In longitudinal sections the following points must be attended to:—

1. The primary insertion of the parts of one or more of the whorls, and whether their position, at a later period of developement, has remained unaltered; or whether the parts of one or other of the whorls are pushed upwards. The formation of a disc, the origin of appendicular organs, the developement of hairs, &c., must also be attended to.

2. The developement of the ovary; whether one cavity or many cavities are formed in the apex of a cone, the cone being primarily flat, but afterwards vaulted; how such an ovary subsequently develops itself; and whether it grows at its apex or at its base—how the stigma and the style are formed, what is the nature of the placentæ, &c.

3. The connexion of the canal of the style with the hollow of the ovary: this connexion can often only be clearly understood by tracing the developement of the flowers; a comparison of good longitudinal sections in different stages of growth will leave no doubt as to the nature of the connexion. The developement of the ovule is treated of in connexion with the origin of the embryo.

The use of the words "adhesion," or "growing together," in speaking of the union of the parts of flowers, as, for instance, in the case of the petals of gamopetalous flowers, frequently gives rise to erroneous ideas. The petals or sepals, which at first appear as separate parts, do not subsequently *grow together* at the bottom, but in the course of the development which takes place at their base, the *separation* subsequently ceases; it would, therefore, be more correct to speak of petals as *not separated* than as *grown together*. A true example of growing together does, however, take place in the stigmas of the

Apocyneæ and Asclepiadeæ, in which cases the two stigmas of each ovary, which are at first completely separated, grow together and form one united stigma.

On the method of tracing the developement of the Embryo.—In order to be at all successful in carrying on this most difficult of all anatomico-physiological investigations, it is necessary, in the first place, to be thoroughly acquainted with the construction of the ovary, the style, and the stigma of the plants to be examined, as well as with the developement of their ovules; it is necessary, at least in some plants, to examine attentively the canal of the style of a flower which has not shed its pollen, as well as the canal of the style of a flower to which the pollen has been applied by the observer himself, in order to become acquainted with the course of the pollen-tube, and the changes to which it gives rise in the canal of the style. Moreover, the condition of the ovule and of the embryo-sac at the period of flowering, before any pollen-tube has reached the ovule, must, in all cases, be fully examined, and the contents of the embryo-sac must be most carefully attended to, since it is only by these means that it is possible to form a correct judgement as to the changes subsequently produced by the agency of the pollen-tube.

In order to trace the course of the pollen-tubes from the stigma into the hollow of the ovary, the best plan is for the observer himself to apply the pollen to the flowers. One or more of the flowers should then be examined daily, by taking thin longitudinal sections from the middle of the style and ovary. By this means we ascertain the time which the pollen takes to protrude the tubes and press them into the ovary. Besides making good longitudinal sections, it is often advantageous to separate the walls of the canal of the style from one another by the aid of the simple microscope; in which case a large bundle of pollen-tubes may often be seen intermixed with the cells of the conducting cellular tissue, and this bundle of tubes may not unfrequently be traced into the hollow of the ovary by the help of a needle under the simple microscope. In plants with long thin styles, which soon wither, the attempt to follow the course of the pollen-tube without interruption

will seldom be successful, but in plants with short fleshy styles it is frequently not difficult. The Orchideæ are the most favourable plants for this purpose. If the style of the flower of an Epipactis, to which the pollen has been applied about eight days previously, be examined in the manner above mentioned, the observer will be surprised at the extraordinary number of pollen-tubes, and he will easily be able to trace them in large strings, even as far as the ovules. Viola tricolor and Ribes nigrum and rubrum, are also good plants for the purpose; in the case of the former plant, withered flowers may be taken, and branched pollen-tubes will not unfrequently be met with. These branched pollen-tubes are found, even more frequently, in the Ænothera muricata. No definite mode of proceeding can be given for tracing the developement of the ovule, which must be regulated by the number and arrangement of the ovules in the ovary. Sometimes the transverse section, sometimes the longitudinal, will be most serviceable. The first appearance of the nucleus of the ovule out of the tissue of the placenta, in the form of a conical cellular little excrescence, the origin of the coatings of the ovule in the shape of circular enveloping folds around the nucleus, the slight contemporaneous bending of the ovule, and the appearance and nature of the embryo-sac within the ovule, must all be noticed. In the Hippuris and Myriophyllum, the ovules are without integuments, and are anatropal. They are also provided with a vascular bundle in the naked nucleus. In Thesium, the nucleus is also naked, but has no vascular bundles. As specimens of plants, the ovules of which are provided with integuments, may be mentioned Juglans, Taxus (in which the ovule is orthotropal), Impatiens, and the Rhinanthaceæ. In the latter, the ovule is anatropal, and the embryo-sac is extended into sac-shaped prolongations, which lie in the parenchyma of the integument. In Hydrocharis and Polygonum, Viola, Ænothera, and the Orchideæ, the ovules have two integuments. In Polygonum, the ovule is orthotropal, and in the Orchideæ, anatropal. Most ovules, at the period of flowering, are so large that they may be detached, placed upon the finger, and sections made of them; the direction of the cut

must be particularly attended to. One side of the ovule must first be removed with an excessively sharp concave-sided razor; the ovule must then be carefully turned round with a fine camel's-hair brush, and the other side removed in like manner by passing the razor steadily and slowly through the ovule, so that of the whole ovule there will remain only the middle lamella. The object must not be permitted to become dry during the process of cutting, so that the finger must be kept moist. The lamella, thus prepared, must be immediately placed under the microscope without a covering-glass. It will frequently happen that the section may be improved by a third or a fourth cut, made in the same manner; and although, in doing this, the object is sometimes spoiled, nevertheless superfluous parts may often be successfully removed in this manner. The needle and the simple microscope will also often be of service. When it is possible, it is very desirable completely to detach the embryo-sac of a flower which has not yet shed its pollen. The embryo-sac then appears in the form of a simple cell; but in most cases it is so tender that it is destroyed by the operation, or at least the cells which take their origin in it are spoiled. In this case, it is better to be satisfied with the thinnest possible longitudinal sections, and to study accurately the contents of the embryo-sac, remarking especially the presence or absence of cells therein, and their situation, if any. A solution of iodine is here desirable. The observer must not be satisfied with the preparation of one specimen, be it ever so successful; many sections, and these as perfect as possible, must be taken and compared with one another. It will then soon be seen whether or not a cellular formation is always to be found within the embryo-sac, even before the pollen-tube has penetrated the ovule, and it will also be seen what explanation is to be given with respect to these cells. In Lathrea Pedicularis and Hippuris, endosperm is formed before the period of impregnation.

When the ovule and the condition of the embryo-sac before the shedding of the pollen has been observed, the same mode of proceeding must be adopted with the ovules of the flowers which have shed their pollen. In the Orchideæ, the ovules of

which are very small and delicate, it is not possible to make sections through the ovules. They therefore cannot be used for investigating the origin of the embryo itself, that is, the examination of them can afford no positive evidence, either in favour of, or against, either of the different opinions entertained upon the subject. On the other hand, the entrance of the pollen-tube into the micropyle can be easily observed in these ovules. It is only necessary to remove the ovules of the swollen ovary of an orchis with a needle, which, after impregnation, may easily be detached from the placentæ, and there will often be discovered from one to five pollen-tubes in one micropyle. It will often be necessary to drive out the air by a gentle pressure with the compressorium. The Euphrasia officinalis may well be employed for the same purpose. It is only necessary to tear open with a needle the ovary of a flower which has just withered, and almost every ovule will be found to contain a pollen-tube. The same thing may be seen in the Veronica serpyllifolia, by making thin transverse and longitudinal sections through the ovary of a flower which has shed its pollen. In some plants, the entry of the pollen-tube into the ovule is for many reasons more difficult to observe, partly because that part of the tube which hangs out of the ovule is very quickly absorbed, as is the case in Ornithogalum and Hippuris, and partly because, from the position of the ovule itself, they are cut away in making the section, as is the case in the Ænothera. In these cases, when the specimen is well prepared the pollen-tube is found either inside the micropyle, or, as in Ænothera, at its passage through the nucleus.

Whenever it is possible, the embryo-sac and the pollen-tube should be completely detached, for I am persuaded that this is the only way to arrive at a complete and final solution of the very difficult question under consideration. It is not always possible completely to separate the apex of the embryo-sac together with the pollen-tube which has penetrated it, from the surrounding tissue. It may often be effected in Canna. In this plant I have often drawn out the pollen-tube uninjured from the embryo-sac, and found the end of the tube already swollen; in Taxus I have done the same with pollen-tubes which had

already developed cells in the corpusculum. The Personatæ are in every case the most favourable plants with which to institute this most important investigation, especially those plants in which the apex of the embryo-sac never becomes filled with cells, and where the nature of the ovule permits of an entire separation of the apex of the embryo-sac. Having made many trials, I can recommend for this purpose the Lathræa squamaria, the Pedicularis palustris, and particularly the Pedicularis sylvatica. I have always been very successful with the Lathræa and the last-named plants. The peculiar construction of the ovule itself, with which the observer must make himself fully acquainted, is, in the plants above mentioned, particularly favourable for the investigation now under discussion; and the form of the ovule will afford a guide as to the direction in which the section must be made. The middle lamella of the ovule, when obtained in the manner pointed out above, must first be placed under the compound microscope, and examined on both sides with a power of about 200 diameters. The lowest eye-piece should alone be used in this examination.

When it is thought that the section may be improved, the side of the section and the particular point to be operated upon must be noted, and the lamella cut accordingly. The specimen must then be again examined, and when the section is considered satisfactory, it should be brought under the simple microscope, with a magnifying power of from 15 to 30 diameters, and the parenchyma surrounding the apex of the embryo-sac removed with the needle. Although the observer may carefully follow the above directions, it will seldom, if ever, be possible, at least with the Lathræa, to separate completely and without injury the whole of the embryo-sac and its prolongations from the surrounding tissue. In tracing the developement of the embryo, it is sufficient if the apex of the embryo-sac is completely separated, so that the observer may be able fully to study the relation of this apex to the pollen-tube which has penetrated it; and with a little perseverance and dexterity this separation of the apex may generally be successfully effected. In Pedicularis and Lathræa there will seldom be found to be any considerable portion of the pollen-tube on the outside of the embryo-sac; it

very soon becomes softened and dissolved in the micropyle. In Lathræa two pollen-tubes will often be met with in the cell-less apex of the embryo-sac; of these one only reaches the endosperm in order to become developed into the embryo. These pollen-tubes are frequently closed above, and cannot always be recognised as the remains of the portions of the pollen-tubes which are found in the micropyle. On the other hand, by accurate adjustment, correct illumination, and careful examination of the specimen on both sides, it may always be seen that the sac which lines the cell-less apex of the embryo-sac, and which, reaching to the endosperm, becomes the embryo, can never originate inside the embryo-sac, but must have penetrated into it from without, because the upper end of it, which is generally round and closed, always projects, often very prominently, above the apex of the embryo-sac; and, moreover, at the place where the pollen-tube has penetrated, there may be observed, in most cases, a decided introversion of the membrane of the embryo-sac, caused by the penetration of the pollen-tube. By preparing a great number of specimens in the manner above mentioned, I traced the origin of the embryo in Lathræa and Pedicularis from the formation of the first cell in the interior of the pollen-tube to the appearance of the two cotyledons. When the young embryo has developed a few cells, the endosperm should be removed; the observer will then be easily and certainly convinced that the first cells of the embryo originate in the interior of the sac above mentioned; and if he has previously satisfied himself of the identity of this sac with the pollen-tube, no further question can be raised as to the origin of the embryo, that is to say, as to the origin of its first cells in the interior of the pollen-tube.*

The importance of the question, and the great difficulty of

* It may be well to caution the reader that Dr. Schacht's views as to the origin of the embryo are by no means generally received. It is stated by a writer in the last number of the 'Microscopical Journal,' that Dr. Schacht and Dr. Hofmeister, the advocate of a contrary theory, met to compare specimens, and parted *mutually unconvinced*. Unger's views upon the subject are given in the Appendix to this translation, and the reader may also refer to Hofmeister's work "Die Entstehung des Embryo der Phanerogamen," and to "Mohl on the Vegetable Cell," translated by Henfrey.—Tr.

its solution, have induced me to enter into the above details; it was particularly necessary to lay down a complete and definite mode of proceeding, since the methods hitherto attempted by other observers are, in my opinion, insufficient. I place no weight, for example, upon the examination of the Orchideæ; the cells of the integuments in these plants are too apt to mislead. The case is not much better with any other plants which do not admit of the separation of the apex of the embryo-sac; such plants I should never recommend to be employed for this investigation, because I consider the complete removal of the apex of the embryo-sac to be the first and most indispensable requisite for a solution of the above question. I think that this is the only way in which it is possible to obtain correct ideas of the true relation of the pollen-tube to the embryo-sac.

Figs. 2, 3, and 4 are explanatory of the matter just treated of. Fig. 2 represents a very perfect longitudinal section through the ovule of Lathræa squamaria at the time of flowering. The nucleus of the ovule has been long since absorbed by the embryo-sac; the embryo-sac has peculiar prolongations (*a*) and (*b*) at either end, which sink deep into the single integument, nay, afterwards even break through it, and project into the hollow of the ovary. The middle part of the embryo-sac becomes filled with endosperm at an early period; the other parts of it never contain cells. The pollen-tube passes through the cell-less apex, which is connected with the front prolongation (*a*), and when it reaches the endosperm, swells and forms the embryo; in this flower many pollen-tubes are often found in the micropyle; (*c*) is the hilum of the ovule.

Fig. 2.

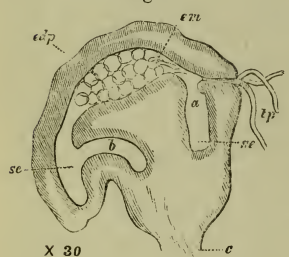


Fig. 3 represents the apex of the embryo-sac of Lathræa squamaria prepared in a perfect and uninjured state, drawn from a perfectly fresh specimen. (*a*) is a part of the front prolongation (see Fig. 2). Two pollen-tubes have penetrated the embryo-sac, one has already swollen into a rudimentary embryo

within the endosperm, the other has penetrated but not developed itself. Both pollen-tubes are seen protruding far out of the cell-less apex of the embryo-sac. The outline of this apex on the upper side is seen distinctly. On the under side it appears as a less defined line, which stands out clearly when the adjustment of the microscope is altered. Both pollen-tubes are somewhat swollen in the parts which project from the embryo-sac; they pass off by degrees into a thin ragged end. The part of the pollen-tube which lies in the micropyle is generally so much softened that it is not possible to detach a large piece of it; the pollen-tube which has penetrated the embryo-sac is frequently broken off at the end which projects from the embryo-sac. Two pollen-tubes are often found in the embryo-sac of Lathræa, but one only developes an embryo.

Fig. 3.

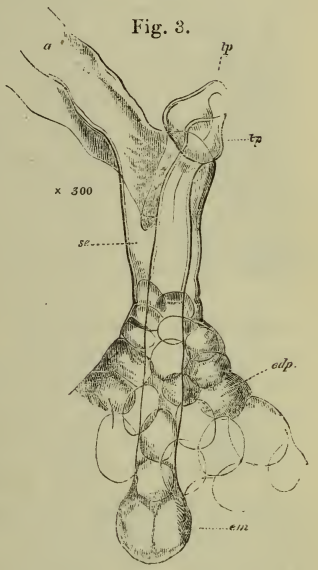
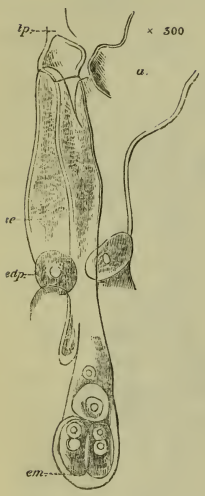


Fig. 4 represents a preparation quite as perfect as that shown in fig. 3, also drawn from a perfectly fresh specimen. (*a*) is the front prolongation of the embryo-sac, into which only one pollen-tube has entered, which projects for some distance out of the embryo-sac, and is broken off. The outline of both sides of the embryo-sac is seen. From the appearance of the outline of the under side, it would appear that the membrane of the embryo-sac was pushed inwards by the entrance of the pollen-tube. The pollen-tube, after having reached the endosperm (which is not represented in this fig.), has formed many cells; which are the foundation of the embryo. The cytoblast has already become divided in the two lower cells. Over these young cells the pollen-tube itself forms a small recess. Specimens such as those represented in figs. 3 and 4 may be kept

in chloride of calcium for a length of time without becoming deteriorated in any way, so far as relates to the pollen-tube and the embryo-sac.

Fig. 4.



Observations on the embryo will be more certain and valuable in proportion as the separate steps of the developement of the embryo are more fully and completely followed out. I recommend the observer not to hurry himself, since one perfect observation is far more valuable than ten imperfect ones. In dicotyledonous plants, the first appearance of the two cotyledons will be seen in the form of small excrescences upon the originally round rudiments of the embryo. The formation of these cotyledons, and of the plumule between them, must then be observed, and particular attention must be paid to the condition of the embryo in the ripe seed, to its position and form, to the presence or absence of albumen (which in Nymphæa is double), to the changes which take place in the integument by the absorption or thickening of the cells, &c. The contents of the cells of the albumen must be tested with reagents. The testa of the seed, which is generally formed by the integuments, frequently exhibits cells very much thickened.

The embryo of monocotyledons presents far more variety in its form and in the arrangement of its parts than the embryo of dicotyledons. An accurate investigation of the former, combined with an account of its mode of germination, is in many instances much to be desired. In investigating the germination both of monocotyledons and dicotyledons, the first thing which is necessary is an accurate investigation of the embryo before germination, so as to ascertain whether the vascular bundles are then already present, and what course they take, and whether the rudiments of leaves are to be found in the plumule or not. The progress of the germ must then be watched at short intervals, and attention must be given to the growth of

the axis, that is, the stem and root, as well as to the growth of the leaves, and to the distribution of the vascular bundles. Thin transverse and longitudinal sections are here necessary.

On the method of tracing the developement of the Cell.—In tracing the developement of the cell, far less skill in preparation is generally requisite than in tracing the developement of the parts of plants, but nevertheless the inquiry is a most difficult one. The reagents are in general of little service here. Iodine and sulphuric acid have too strong an effect upon very young cells. The cell-contents generally coagulate immediately. A solution of iodine, or of chloride of zinc and iodine, a diluted solution of potash, or diluted acids, are preferable. A large number of observations, and an accurate appreciation of their results, are of the greatest importance.

The developement of free cells may be studied in the spores and gemmæ of Cryptogams, and in the pollen of Phanerogams. In order to examine the developement of spores and pollen, it is necessary to make thin transverse and longitudinal sections through the situs of the spores, and through the anthers, in the earliest stage of their developement. In the mosses and liverworts, the sections must be made at such a period as to exhibit only one row of the primary mother-cells in the circumference of the columella. In the ferns the examination must begin at the time of the appearance of the Sporangium in the form of a simple cell: in the Phanerogamia young anthers must be taken at the period of the appearance of a single row of mother-cells in each chamber of the anther. When the size of the rudimentary spores or of the young pollen has been ascertained, their diameter measured, and their mass observed, the subsequent examination must be carried on step by step. The spike is a very favourable style of inflorescence for examining the pollen. The investigation must here be commenced with the buds of the summit of the apex, and must be carried downwards by degrees without passing over any part. In very young stages of a plant transverse sections are best made through the whole bud; the anthers which have been thus cut through must then be detached by the aid of the simple microscope, the disposition of the mother-cells in the chamber of the

anther must be carefully observed, and these latter cells must then be isolated by removing the surrounding parenchyma with the needle. In examining the mother-cells, both of pollen and spores, the attention must be directed to their size and to the nature of their walls; it must be ascertained whether these walls are double or single, and the manner in which they are acted upon by iodine, and by the iodized solution of chloride of zinc, must be observed. Particular attention must also be paid to their contents. Inquiry must also be made as to the existence of a cytoblast, and whether the same is central or attached to the walls, whether it exhibits a tendency to divide, or whether division has already begun to take place; moreover, the number of its nucleoli and the distribution of the mucilage, which is usually of a granular nature, over the cell-wall, and the relation of this mucilage to the cytoblast, must also be observed. Lastly, it must be seen whether these primary mother-cells become themselves the mother-cells of others, or whether the spores and the pollen-grains are developed within them. In Anthoceros the latter is the case; in the anther of Meriolix the mother-cell forms, in the first instance, new generations of other mother-cells, before the four pollen-grains are developed, which developement takes place in the last generation; the number of these generations of mother-cells can hardly be determined with accuracy, since there is a deficiency of reliable observations relative to the successive steps of the developement. In some cases difficulties arise from the nature of the contents of the mother-cells and of the cells produced by them. These contents are often granular. Inasmuch, however, as the observations which we are now considering are carried on, not with one but with a great number of cells, those specimens can be selected which appear to be most favourable for the investigation. The use of a diluted solution of potash is sometimes advantageous. The examination must be carried on until the formation of the spore or pollen is perfect. The use of iodine, and of the iodized solution of chloride of zinc, is very important for ascertaining the chemical nature of the cells and their contents. A maceration of the whole anther or young spore, according to Schulz's method, and a subsequent treatment of them with the iodized

solution of chloride of zinc, might perhaps be beneficial. The observations previously made with respect to the perfect flower are applicable to the treatment of the perfect spore and pollen. The developement of Gemmæ must always be traced from the primary cell, whether they be formed in peculiar organs, or at certain definite or indefinite positions on the plant. The developement of the Gemmæ of Blasia affords much interesting matter for observation; in this case thin longitudinal sections must be made through the rudimentary Gemmæ. Similar Gemmæ are developed in winter and in spring on the underside of the youngest leaves of Jungermannia anomala; in this case thin longitudinal sections must be made through the middle of the stem. The Gemmæ produced by phanerogamous plants, for instance on the leaves of Bryophyllum, should also be considered; an accurate account of their developement from the primary cell would be of very great value. It would also be of much importance if the Gemmæ of cryptogamous plants were examined with reference to their subsequent developement into perfect plants, and if this developement were considered in connexion with the history of the germination of plants of the same species.

In the examination of close tissues (geschlossenen gewebe) much difficulty generally arises from the diminutive size of the cells, and the quantity of granular matter within them. For examining this tissue, the apex of the axis, *i. e.*, of the stem as well as of the root, the base of the young leaves, and especially the primary cell-formation in the embryo-sac, should be selected. The developement of the leaves of Liverworts and of Sphagnum appears also to be favourable for the observation of it.

Many opinions prevail upon the subject of the formation of cells; it has sometimes been assumed that, besides the formation of cells within mother-cells, a formation of cells takes place by the division of primary cells: doubts have also been raised as to the existence of the cytoblast, and its relation to the formation of cells. I may, therefore, be permitted shortly to state my opinion as to cell-formation, which opinion is grounded upon many carefully-conducted observations.

I believe that there is only one kind of cell-formation, that

is, a formation of young cells within mother-cells. I believe that a young cell is present in every case where the division of a primary cell appears to take place; but that the wall of the young cell is so delicate that it cannot be distinguished. I believe that no cell-formation ever takes place without the presence of a cytoblast; the cytoblast originates in the division of an older cytoblast, which latter generally falls into two pieces. In Anthoceros the new cytoblast becomes divided in the same manner. The influence of the cytoblast upon the origin of the young cell is very clearly seen in Anthoceros. A mass of mucilaginous threads pass off from the periphery of the mother-cell to the cytoblasts; the mucilaginous investment of the mother-cell is finally divided into four parts, into four closed mucilaginous cells, each of which has its cytoblast; the layer of cellulose which is devoid of nitrogen is afterwards developed over the primary nitrogenous membrane. In the embryo-sac of phanerogamous plants also, the cytoblast appears first; this is surrounded by a more or less extensive zone of mucilage, out of which the primary nitrogenous covering, *i. e.*, the primordial utricle, appears to be formed, and over the primordial utricle the covering of cellulose is formed at a subsequent period.

In the embryo-sac the cell-formation always commences in the periphery of its membrane; it would appear, therefore, that the nitrogenous covering of the latter, that is, the primordial utricle, is active in cell-formation. I must, therefore, admit two modifications of cell-formation; the one where the primordial utricle of the mother-cell divides into as many parts as there are young cells originating from the mother-cell; the other where no direct division of the primordial utricle takes place: in both cases the cellulose covering is formed subsequently to the primordial utricle. From the circumstance of the primary primordial utricle becoming divided into a definite number of secondary primordial utricles, it appears probable that the cellulose covering of the mother-cell is expended in the formation of new coverings of cellulose for the young cells; the cellulose of the mother-cell becomes softened to a gelatinous consistency contemporaneously with the division of the pri-

mordial utricle; at a subsequent period this cellulose entirely disappears: moreover, in every case, new cellulose for the thickening of the cell-wall is formed from the primordial utricle of the young cell. At what period this formation of cellulose takes place is a question. The swelling of the mother-cells at the period of the formation of the young cells has, in my opinion, given rise to the notion of the division of the former.

CHAPTER VI.

EXAMPLES OF THE DEVELOPEMENT OF FLOWERS.

In the figures which occur in this chapter, as well as in the rest of the work, the following abbreviations are used:—

THE FLOWER.	
anth.	Anther.
bract.	Bract.
filam.	Filament.
gemm.	Ovule.
germ.	Ovary.
m. poll.	Pollen-mass.
pet.	Petal.
sep.	Sepal.
spermoph.	Spermophore.
stigm.	Stigma.
styl.	Style.

THE OVULE.	
ch.	Chalaza.
em.	Embryo.
edp.	Endosperm.
i. e.	Integumentum externum
i. i.	Integumentum internum.
i. s.	Integumentum simplex.
nc.	Nucleus.
se.	Embryo-sac.
tp.	Pollen-tube.

As a specimen of a regular flower, that is to say, a flower in which each whorl consists of the same number of parts, I have chosen Asclepias, because the filaments, the anthers, and the ovary, exhibit peculiarities of construction arising in the course of developement.

As specimens of irregular flowers, I have chosen Stachys and Salvia, because in them the stamens are deficient in number; and Cleome, because in this flower the whorl of stamens consists of more parts than the two preceding whorls.

Asclepias Syriaca.—Fig. 5 represents the developed flower of Asclepias Syriaca, seen from above.

Fig. 5.*

Fig. 6.

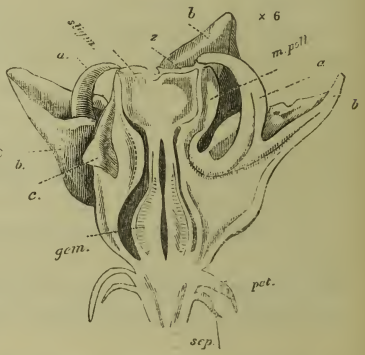


Fig. 6 represents the same flower bisected longitudinally, *a*, *b*, *c*, represent the same parts in each of the figures; *a*, and *b*, are the appendages of the filament, which will be mentioned hereafter.

Fig. 7.

Fig. 7 represents the same flower seen from the side.

The first rudiments of the flower appear in the form of a small round cellular excrescence in the axil of the young bract; shortly afterwards, five small protuberances appear in a circle upon this excrescence; these are the five leaves of the calyx,

* In this Figure "× 6" implies that the flower is magnified six diameters; and so of the other figures where the sign occurs.

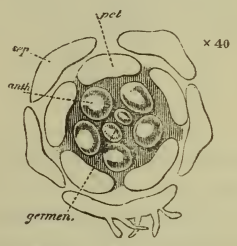
i. e., the sepals, and are seen in fig. 8, which represents a transverse section of the rudimentary flower in a very young state.

Fig. 8.

Fig 9.

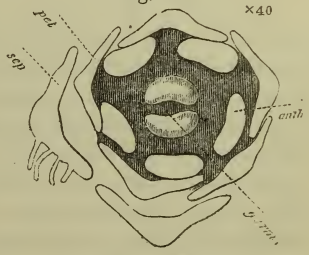
A longitudinal section made at this period is represented in fig. 9, and exhibits the apex of the axis in the form of a slightly-arched surface surrounded by the sepals. After a short time, the five petals appear in the form of five protuberances alternating with the sepals, which, in the mean time, have become larger; soon afterwards, a third whorl makes its appearance, consisting of five little excrescences alternating with the petals; these are the anthers, and are to be seen in fig. 10. Up to this point the apex of the axis has retained the form of an arched surface; now, however, two small protuberances proceed from it which are the first rudiments of the ovary. These two small protuberances are seen in fig. 10 within the anthers.

Fig. 10.



All the parts of the flower are now established, and continue to be developed together. There is nothing further requiring particular notice in the calyx and petals; the five stamens and the two pistils now alone arrest our attention. The anthers, which, in their origin, were in the shape of round excrescences, appear soon afterwards, upon making a transverse section of them, to be elongated, and to be of the form shown in fig. 11, which repre-

Fig. 11.



sents a transverse section of a more advanced flower, three of the sepals being omitted. The longitudinal section, fig. 12, shows that they are inserted somewhat higher up than the petals. If a transverse section be made shortly afterwards, the vascular bundle of the connective may be seen. Fig. 13

Fig. 12.

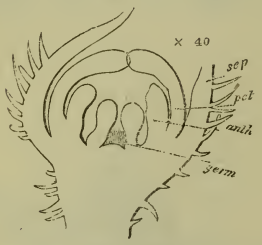
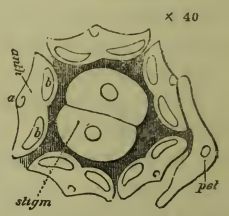


Fig. 13.



represents such a transverse section, the sepals being omitted, and only one petal drawn; (*a*) represents the vascular bundle, and (*b*) (*b*) the rudiments of the two loculi of the anthers. Figs. 14 and 15 represent longitudinal sections which also

Fig. 14.

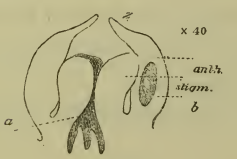
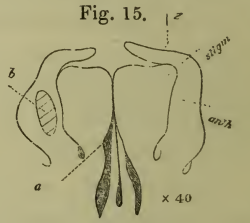


Fig. 15.

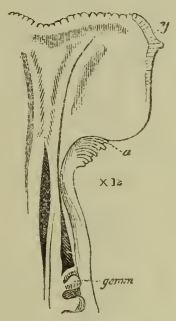


show at (*b*) in each figure, the origin of the loculi of the anthers; the apex of the anthers (*z*) is spread out, as appears in both figures, flatly over the stigmas; the filament of the anther is as yet simple without any appendages. The sepals and petals are omitted in figs. 14 and 15.

Let us now turn to the pistils, which we left at fig. 10, in the form of two small wart-like excrescences surrounded by the anthers. In fig. 11 we have seen them as two half-moon-shaped organs with their edges turned towards each other. Fig. 12 exhibits a longitudinal section of them answering to

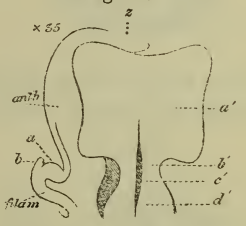
the transverse section in fig. 11. As the developement proceeds, the curvature of each rudiment of the ovary increases, the two edges approximate more and more at their base, and at last turn completely inwards; the upper parts of the pistil on the other hand do not become curved in one mass with the ovaries; in fact, the stigma does not become curved at all; the style, therefore, does not terminate as in other plants at the apex but underneath the stigma.

Fig. 16.



The longitudinal sections figs. 14 and 15, exhibit at (*a*) the place where the style ends; at a later period there is formed there a conducting tissue consisting of long papillæ. Fig. 16 represents a longitudinal section through the upper part of the pistil of a flower almost developed; (*a*) is the place under the stigma where the pollen tubes enter the canal of the style; (*γ*) is the secreting portion of the stigma, and will be referred to hereafter. The figure only shows half the pistil. In figs. 14 and 15 the stigmas of the two pistils are not yet grown together; a little later they become united as shown in fig. 17, which represent a longitudinal section with the calyx and petals removed, and showing only one anther. Transverse sections through the ovaries made at this period at different heights, as, for instance, at the points *a′ b′ c′* and *d′* in fig. 17, exhibit the circumstances which have been heretofore stated with respect to the developement of the rudiments of each individual ovary. Fig. 18 (*a′ b′ c′ d′*) represents four transverse sections through the two ovaries of a young flower, at different heights, such heights being shown in fig. 17 by the same letters of reference;

Fig. 17.



d' is the lowest part of the two young pistils, + + is one of the vascular bundles at the bottom of the flower; ×, on the other hand, which recurs at c' b' and a', is the vascular bundle of the pistil itself. At d', in fig. 18, the two edges of each pistil turn completely inwards; at a later period they form the placentæ, shown in fig. 19, which represents a transverse section of the lower part of an ovary at the time of flowering; at c', in fig. 18, the curvature is still present, and through it the canal of the style (x), takes its origin; at b' in fig. 18, the end of this canal is seen underneath the stigmas, and at a' the last trace of the canal of the style (x) is seen within the two stigmas which have now grown together. About the time when the two stigmas grow together a slight excrescence arises underneath the anthers, which are now to a certain extent developed; in the axil of this excrescence a small protuberance arises (*see* fig. 17, *b*, *a*). At this spot a peculiar bending in the vascular bundles of the anthers takes place, which bending increases perceptibly with the further developement of the appendage above mentioned.

Fig. 18.

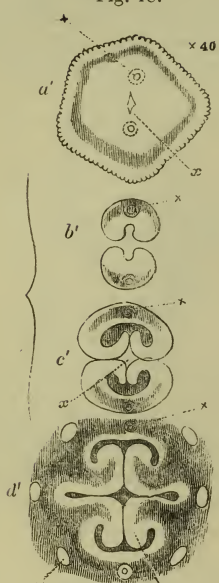
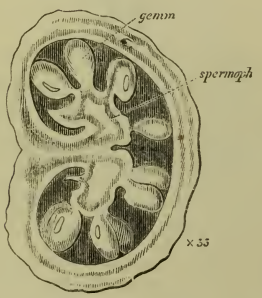


Fig. 19.



(*a*) and (*b*) fig. 17, subsequently become developed into (*a*) and (*b*) figs. 5 and 6.

We have now reached the full developement of the flower;

the calyx and petals are not grown together, but bend downwards at the time of flowering (figs. 6 and 7); the filaments of the anthers, on the other hand, are joined one with another at their lower extremities to a fleshy ring; besides the two appendages above mentioned (*a* and *b*, fig. 6), there appears on each side of the anthers a further wing-shaped expansion of the filament; in fact, two wings, belonging to different filaments, are placed close to one another (figs. 5 and 6) (*c*), whilst a thin skin-like expansion (*z*) (figs. 5, 6, 14, 15, 17) proceeding from the apex of the anthers, covers the stigma. The anther is from the first bilocular (fig. 13, and fig. 20). Fig. 20 represents a transverse section of the upper part of a perfect anther. Owing to the peculiar form of the stamens very different appearances are presented, according to the height at which such a section is made. (*a*) is the vascular bundle of the connective (*b*), (*b*) the two chambers of the anther; no separate pollen-grains are developed in the two loculi, as is the case with most other plants, but they each contain a pollen-mass covered with a leathery skin. I was unable to follow the developement of the cells of these pollen-masses with sufficient accuracy on account of the granular nature of their contents; the mother-cells at a later period lie in rows (fig. 14 (*b*), and fig. 15) (*b*). In fig. 21 at (*a'*) the primary mother-cell is represented, as it appears in a transverse section through a very young anther surrounded by the small adjoining cells; two cytoblasts, and a line between them, show the origin of two new cells in the mother-cell. In fig. 21 (*b'*) is shown part of a thin transverse section through

Fig. 20.

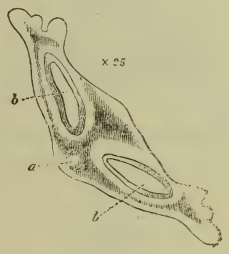
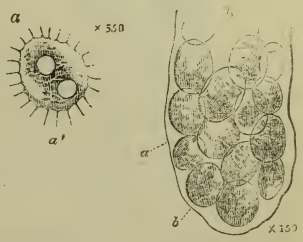


Fig. 21.



the perfect pollen-mass, (*a*) being the leathery skin surrounding the pollen-cells (*b*). I consider the leathery skin to be a secretion; it becomes of a rich claret colour upon the application of concentrated sulphuric acid; the substance which is diffused here and there through the pollen-cells becomes coloured in like manner.

The stigma is pentagonal: at five particular points of it the epidermis is developed into the form of papillæ (Fig. 16 *y*). These places which, in a transverse section, assume the form of channels, secrete a fluid which gradually hardens, assumes a definite form, and becomes converted into the so-called gland which unites the two pollen-masses.

Fig. 22.

In fig. 22 is represented a small part of a thin transverse section through the stigma of a flower almost developed; *y* is the secreting epithelium, consisting of long thin papillæ; *x* is the hardened secretion, or so-called gland (also in transverse section), and which is united to the papillæ. Fig. 23 represents two pollen-masses belonging to different anthers united by the so-called gland (*x*) and by the caudiculi.

Fig. 23.

The secreting surface of the stigma is extended in a modified form as far as the place where the chamber of the anther opens: the secretion from this surface forms the caudiculus which is protruded by the so-called gland, and which bears the pollen-masses. The position of the secreting surface of the stigma at (*c*) (fig. 5), gives rise to the peculiar circumstance that each one of the so-called glands unites two pollen-masses produced from two different anthers.

In tracing the developement, it appears most clearly that that which has been called a gland is not really a gland, but is a true secretion. Upon making very thin transverse sections through the stigma at different periods of its growth, the secre-

tion will be found to be at one time fluid, and at another half fluid and already hardened on the outside, whilst additions are made to it by new secretions from the surface of the stigma itself. The apparently cellular structure of the complete mass is caused by the impression of the secreting cells; the uniform nature of this mass is seen by making a thin section through it, and treating it with caustic potash. The so-called gland of the Asclepiadeæ is therefore something quite different from the rostellum of the Orchideæ, which really consists of cells, and is a true gland, that is, an organ which secretes a particular fluid.

The Asclepiadeæ, as was known long since, can only be impregnated by insects, or artificially. The place where the pollen-tubes can penetrate lies close under the stigma, fig. 16, (*a*), and is shown by long papillæ; the epithelium of the filament of the anthers also forms a secretion at this place. The two ovaries of the perfect flower remain completely separated down to their base, although their stigmas adhere to one another; upon making a transverse section the placentæ of each ovary appear spread out on both sides, and bearing many rows of ovules, as represented in fig. 19.

Fig. 24.

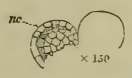


Fig. 25.

Fig. 26.

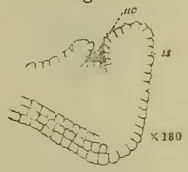
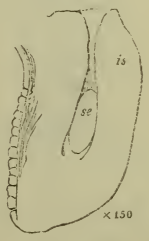


Fig. 27.



The ovule has only one integument, and the nucleus, which is but slightly developed, disappears at an early period, being absorbed by the embryo-sac. It is not visible at the time of flowering. Figs. 24, 25, 26, and 27, represent different stages of the developement of the ovule.

The pistil increases in growth at its apex; the two stigmas are formed last; they are originally separate, and become united

at their apex at a subsequent period, so that the growing-cells must be situated at their apex (fig. 15 and fig. 17). The anthers, on the other hand, grow at their base; the very beautiful appendage (*z*) is already present when the chambers of the anthers are forming (figs. 14, 15, 17, and 6). The appendages (*a*) and (*b*) of the filaments, on the other hand, appear at a much later period; the increasing curvature of the vascular bundle, which takes place contemporaneously with the further developement of the above-mentioned appendages, indicates a further developement of the vascular bundle at this place.

The two stigmas of the separate pistils are united by a real *adhesion*, or *growing together*. In other plants, the parts which appear to have become grown together, have not really become so, but only apparently, the fact being that the original separation of the parts is not continued in their subsequent growth.

Stachys Coccinea.—The rudiment of the flower appears, as in the case of Asclepias, in the form of a small cellular cone in the axil of the bract. A short time afterwards five protuberances appear upon it, which are the five sepals. Fig. 28 represents a longitudinal section of the first rudiments of the flower. At a later period a second circle, consisting of similar excrescences, is perceived; these are the petals, which alternate with the sepals. A third whorl now follows, which, however, consists of only four elements, alternating with the rudiments of the petals. These are the anthers, the fifth of which has not begun to be developed. Fig. 29 represents this stage of the flower seen from above. It is very probable, notwithstanding the pains I have taken, that that condition of the plant which would exhibit the rudiment of the fifth anther arrested in its growth, may have escaped my attention; in Salvia nivea I was so fortunate as to meet with the three

Fig. 28.

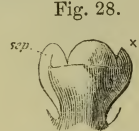
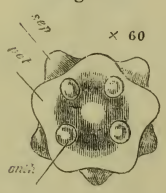


Fig. 29.



arrested anthers in their rudimentary state. Fig. 30 represents a transverse section of a very young bud of Salvia nivea (x), being the traces of the three abortive anthers. The parts of the calyx in Stachys coccinea, as well as of the corolla, are separated at first; they do not afterwards grow together, but the fact rather is, that the separation ceases during their subsequent developement. In fig. 29 we have seen the petals united at their base. At a somewhat later period the parts of the calyx, as well as of the corolla, are completely united to one another; that is to say, the lower portions of them have never been divided into separate parts from the first period of their growth. The growth of the calyx and corolla is here shown to be analogous to that of the leaves, in the fact that the divided apices have been first formed.

Fig. 30.

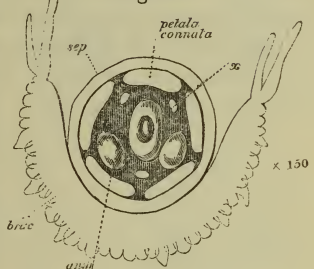


Fig. 31.

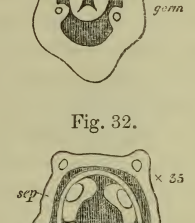


Fig. 32.

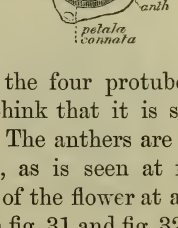


Fig. 31 and fig. 32 are transverse sections of one and the same flower at different heights; at fig. 32 is seen the empty situs of the fifth abortive anther. I cannot confidently state whether the ovary really consists originally of four parts, because the four protuberances are at first so slightly developed, but I think that it is so, both in this flower and in the Borragineæ. The anthers are at first inserted only a little above the petals, as is seen at fig. 33, which represents a longitudinal section of the flower at a period somewhat later than that represented in fig. 31 and fig. 32. The ovary of the Labiatæ as well as of the Borragineæ appears, by fol-

lowing out its developement, to be unilocular, with two parietal placentæ, each of which bears two ovules. Fig. 34 represents a transverse section through a young ovary. From the subsequent unequal developement of the wall of the ovary, which appears to be produced by the perfecting of the four ovules, the four nut-like excrescences originate, which are connected together by the single canal of the style. Fig. 35 represents a longitudinal

Fig. 33.

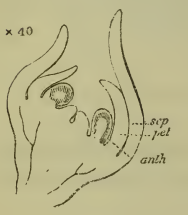


Fig. 34.

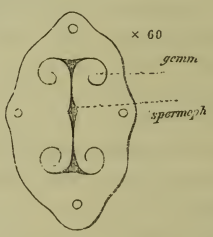
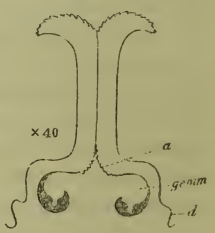


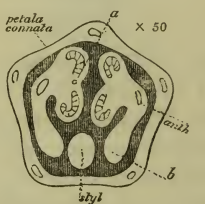
Fig. 35.



section through the young ovary with the style and stigma; (*d*) is the disc. The ovules of the Labiatæ have only one integument. In Salvia and Galeopsis I found prolongations of the embryo-sac similar to those in Lathræa and Pedicularis.

Salvia Nivea.—The first appearances of the developement of the flower are just the same as in Stachys, the calyx appears first, afterwards the petals, and then the anthers. Fig. 30, p. 109, shows a very successful transverse section through the rudiments of the flower, whilst it is still concealed by the bract; the calyx has already grown together, the petals, on the other hand, are still separate; only two of the anthers are as yet visible in the form of large round warts; the three others appear like very small oblong excrescences (*x*): the position of these five anthers, both of the three abortive ones, and of the two which are developed, alternates with the petals; the two anthers which are not abortive produce pollen only on one side. Fig 36 represents a transverse section of the young flower with the bract and calyx

Fig. 36.



removed; the side of the anthers which produces pollen is bilocular; it bursts, when ripe, with a longitudinal dehiscence. Figs. 37 and 38 represent the stamens in process of developement; fig. 39, the two stamens of the developed flower.

Fig. 37. Fig. 38.

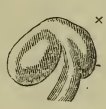


Fig. 39.

Cleome Arborea. — The first rudiments of the flower appear, in the usual manner, in the form of a cellular cone in the axil of the bract; shortly afterwards appear the rudiments of the four sepals, and next to these come the four petals alternating with the sepals. Figs. 40 and 41 represent a flower in a young state seen from above. After this, however, follows a whorl of six elements. Fig. 42 shows a flower at a somewhat later stage than is represented at fig. 41. In fig. 42 the sepals are removed, and only two of the petals drawn. Fig. 43 represents a very perfect transverse section, showing all the parts of the flower. It might, perhaps, be supposed that there were two whorls of anthers, each whorl containing four elements, and that two of the elements of these whorls were abortive; but that this cannot be so is shown by the regular position of the six anthers in one whorl, which always occurs in good specimens. The long-stalked ovary, which is subsequently developed, appears in the form of a solid column; at

Fig. 40. Fig. 41.

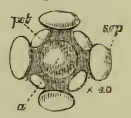


Fig. 42.

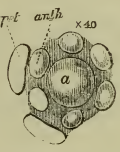
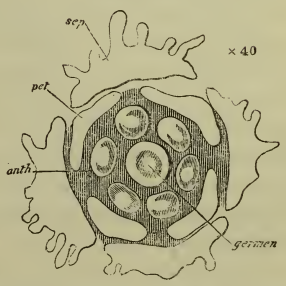


Fig. 43.



its apex there is formed a small depression which at first is very flat; the depression subsequently increases, and the ovary assumes the form of a cup. The edge of this cup afterwards increases in thickness, its walls approximate to one another, and form the stigmas and the style. The ovary is unilocular with two parietal placentæ. The ovule has two integuments; at a later period it exhibits a peculiar curvature; the anther is quadrilocular, but at the time of dehiscing it is bilocular.

Fig. 44 represents a longitudinal section corresponding to the stage of developement, represented at fig. 43. Figs. 45 and 46 represent longitudinal sections of the ovary in different

Fig. 44.

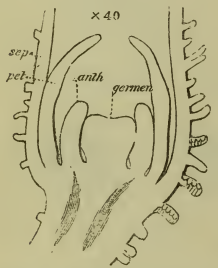


Fig. 45.

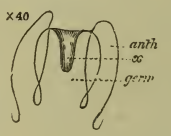
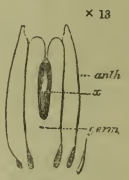


Fig. 46.



stages of developement, the sepals and petals being removed; (x) is the hollow of the ovary. Figs. 47 and 48 represent transverse sections of the ovary in different stages of developement. Fig. 49 represents a transverse section of the young anther; it is

Fig. 47.

Fig. 48.

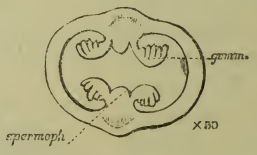


Fig. 49.

quadrilocular; (a) is the vascular bundle of the connective, (b) one of the chambers of the anther, (c) the cellular tissue of the connective, which is absorbed shortly before the dehiscence of the anther.

CHAPTER VII.

ON THE DRAWING OF OBJECTS IN NATURAL PHILOSOPHY GENERALLY, AND MICROSCOPICAL OBJECTS PARTICULARLY.

In all departments of natural philosophy some dexterity in drawing is indispensable; a person who is unable to make drawings of the objects which he examines, and who is obliged to avail himself of the assistance of others in making such drawings, will always work at a disadvantage, because in drawings of objects in natural philosophy two things are necessary; first, dexterity in drawing, and secondly, a thorough knowledge of the object to be represented. The value of the drawing will be in proportion to the amount of this dexterity and knowledge; if the one or the other be wanting, the drawing will frequently be deficient, and sometimes quite useless. Dexterity in drawing comprehends more than a skilful handling of the pencil or brush, and a knowledge and accurate use of colour; these, no doubt, are matters of great importance, but that which is above all things necessary is *a just perception of nature;* the drawing must be a living representation of the object; it should show at a glance that the artist understood the character of the object, and how to render it accurately. To attain to such a perception of nature, the first thing which is necessary is to learn to *see* accurately, which can only be done by a constant and thorough study of nature. In the institutions of Germany and France, a method of instruction, grounded upon the principle of drawing from nature, has been introduced; it is much to be wished that the same method was universally adopted in all schools, from the highest to the lowest. A method of drawing which cultivates the pupil's powers of observation and perception, has, at the same time, a beneficial effect upon the

understanding; the pupil learns whilst he draws to estimate the relative importance of different circumstances; he learns the important laws of perspective, and of light and shade; by the aid of perspective he learns to judge of distances; by means of light and shade he learns the properties of illumination, and its effect upon the appearance of forms and the hues of colours; on the other hand, the person who draws only from copies will at best copy accurately; he will never learn to distinguish what is true from what is false, and he will never have a just idea of nature. I believe that I am fully justified in thus insisting upon the importance of a rational mode of instruction in drawing; I have mentioned how instruction of this nature exercises the eye and developes the understanding. This fact has been long since admitted in mechanical schools; in classical schools, on the other hand, drawing has been too much neglected, yet many a person who has devoted himself to natural philosophy, would rejoice if he had the power of drawing, even to a small extent: it would lighten his studies, and enable him to keep a record of many an interesting occurrence, to the benefit both of himself and of science. It is not necessary to be a great artist in order to be able to give an intelligible representation of an object in nature; it is necessary, as has been already observed, only to *see* and *comprehend* with accuracy. The power of drawing is indispensable to the natural philosopher; by the aid of it many things in nature are seen with greater interest; it leads to a knowledge of art, and affords an agreeable employment for leisure hours.

The *understanding* of any object includes a knowledge of all its parts, and of the relation of these parts to the whole. The *understanding* of an object is therefore quite different from the *accurate seeing* of it; the latter lays hold, in the first instance, upon the characteristics of the object; it affords a general impression of it, but does not trouble itself with details. To *understand* an object the details must be gone into. A *general* drawing of an animal or a plant will, in most cases, be far better executed by a real artist than by a man of science; the former is satisfied, and properly so, with the general impression. He avoids everything which might interfere with that impression.

He only draws that which he really sees. The man of science, on the other hand, unless he be also an artist, which, unfortunately, is seldom the case, is very apt to introduce too much into his drawing, but it is a rule in science, as everywhere else, only to draw that which is actually seen, and to represent everything as it actually appears. In a *general* drawing of an object, *generalities* will be prominent; in a *detailed* drawing, on the other hand, *details* will be conspicuous; in the one case the main point is the *general impression* produced by the object, in the other it is the individual parts in their relation to one another. It is therefore sufficiently clear that if the details of an animal or a plant are to be correctly represented, it is necessary that the observer himself should be able to draw.

In most cases, it will be sufficient for the natural philosopher to be able to draw with a lead-pencil, or what is better, with a brush and Indian ink. Any person who understands drawing can effect a great deal with few appliances. In many cases a knowledge of colours also will be valuable; to attain this knowledge, a man must be something of a colourist. The hues of colours must be studied from nature; the mixing of them will best be learnt from an efficient master, or by long practice. Water-colours are generally sufficient for scientific drawings, and, in fact, are in most cases the only colours which are capable of being used; oil-colours can only be used for drawings of general subjects, and even for these water-colours are generally sufficient: oil-colours require a peculiar knowledge.

Little can be said about the art of using colours. This must be studied in the regular way. It is but seldom that pure colours can be used; it is necessary to learn to mix them, and in order to use them with advantage, the artist must make himself acquainted, as fully as possible, with the peculiarity of each colour; this is of especial importance in the azure colours, such as carmine, burnt sienna, gamboge, sap-green, brown-pink, and bister. If, for example, it is wished to obtain the fiery colour of the pomegranate-blossom, a coat of gamboge is laid on, left to dry, and afterwards painted over with carmine; if, on the other hand, the two colours are mixed together, a different shade of red, and by no means a brilliant one, is obtained. For

particular shades of violet it is also better not to mix the blue and red together, but to use the colours one after the other. In mixing a *green*, Prussian blue should never be used, inasmuch as it becomes darker by age; indigo and gamboge answer very well for this purpose; for a brilliant green, sap-green and brown-pink should be used.

In drawings of general subjects correct shading is essential. Shadows may be very well laid in with neutral-tint, of which there are many different shades. The shadows should be laid in to their full depth, and the colour carried over them afterwards. There are only a few particular colours which are injured by neutral-tint; for instance, the shadow of a bright yellow will appear somewhat dirty if laid in with neutral-tint. The neutral-tint should in this case either not be used at all, or laid in very slightly Indian ink is not adapted for shading, but sepia answers very well for that purpose. Indian ink, however, may be used with great advantage for fine and definite outlines, for which neither neutral-tint nor sepia is adapted. The only thing to be guarded against is making the outline too strong, because, when this is done, the moist colours which are subsequently applied are apt to be made smeary by contact with the outline. When broad lines are necessary, therefore, they should be laid on last, and in the same way the azure colours, when used for very dark shadows, must be laid on last of all. Gum-water, or a little gum-arabic mixed with the colours, are sometimes used for this purpose.

Good drawings require good paper, which must vary according to the description of the drawing. For microscopical drawings in pencil, smooth woven drawing-paper is the best; for some sorts of drawings in colour a less smooth paper should be used; for very large drawings, whether of general or botanical subjects, a granular paper, such as is used by landscape-painters, is preferable. The nature of the paper which is used is by no means an unimportant matter. In order to produce good drawings, the paper must be of a good sort. It is quite a mistake to suppose that it is possible to draw or paint well upon any sort of paper.

For shading with the lead-pencil it is necessary to have many

sorts; for outlines hard kinds only are necessary. For very fine outlines in water-colours the small brushes made of the hair of the marten are particularly desirable; for shading, the brown-haired brushes are better. It is necessary to have six or eight brushes of different thicknesses, and with points of different sizes; for laying on broad shadows very broad-pointed brushes are the best.

The drawing-pencil is generally used for fine outlines, but I prefer the brush. The use of the latter requires more practice, but when facility in the use of it has once been acquired, far better results will be attained, and more rapid progress made with the brush than with the pencil. A microscopical drawing executed in pencil is moreover very inferior. The relative strength and importance of each line can be far better and more accurately rendered with the brush.

It should never be forgotten what a philosophical drawing ought to be; it ought to be an accurate image of nature, but in no respects a *subjective* representation. On this account, as I have said before, I do not value drawings from memory; on the other hand, perfect drawings executed with the skill of an artist are not always to be expected, but only faithful and intelligible drawings.

For microscopical purposes outline drawings are generally quite sufficient; in tracing the developement of the parts of the flower anything beyond this is superfluous; in some microscopical objects the outlines of the cells and their contents are alone important. A person who has never drawn before will, after a little practice, if he sets to work in earnest, learn sufficient to enable him to furnish useful representations of microscopical objects, which are generally nothing more than representations of *surfaces*. The camera-lucida will be found to be of great service.

The drawing of an object as a whole is, on the other hand, far more difficult; in this case the perception of an artist is required. In addition to the circumstances connected with the size and form of the object, it is necessary to attend to its position, to the fore-shortenings which arise from the laws of perspective, and to the way in which the shadows fall. It is

necessary, therefore, in drawing a corporeal object, to arrange the light and the position of the object, so that the latter may be favourably placed for observing its form and external properties; the object must be seen from one and the same point of view, and under one and the same illumination. If one drawing is not successful, two or more should be made of the same object in different positions and under different illuminations.

As a general rule, thin sections are the only objects which are treated under the microscope; corporeal objects are not so often examined, and when they are, it is only with a low magnifying power and with incident light. In drawing such objects, the rules given above with respect to the drawing of an object as a whole, will apply. It will be necessary to select the best position of the object with respect to the light, and the shadows and the perspective must be attended to. When higher magnifying powers and transmitted light are used, the objects thus examined are generally only *surfaces*: in this case shadows will only be perceptible at the boundaries of the object, or of the cells; the thinner the section, and the more directly the light passes through it (as, for example, from a plane mirror), the more faint will the shadows be. With obliquely-transmitted light the shadows will be observable, and on that rests the whole value of this mode of illumination. When such a shadow is seen in the microscope it must be represented in the drawing. In making microscopical drawings, or any other kind of drawing in natural philosophy, it must be laid down as a rule that everything which is seen must be drawn as it actually appears, provided only that what is drawn is ascertained to belong to the object under examination. By observing the shadows, which are especially well-defined in all wood-cells, we distinguish the depth of the cells. The degree of sharpness, breadth, and darkness, of the individual lines in the drawing of the figure, is of even more importance than the casting of the shadows. In making careful drawings it will often happen that the observer will notice matters of the utmost importance which might, perhaps, have otherwise been overlooked; by drawing also he will become far better acquainted

with the particular details of the object, he will not be so easily satisfied with the specimens which he prepares as he would otherwise be; he will be anxious that such specimens should be of the best kind, and the completeness and value of the drawing, and, in fact, of the whole observation, will increase in proportion to his anxiety for success.

When a camera-lucida is used for drawing, it frequently happens, especially with high powers, that by an alteration of adjustment the image is somewhat shifted, the drawing must then be shifted in like manner before anything further is done to it, so as to make the image and the drawing again coincident. The same thing must be done when the object is moved, in order to bring other parts of it into the field of view; a little practice will render the observer familiar with these little devices. It is very necessary to become accustomed to keeping both eyes open during an observation; if the microscope is much used, the same eye should always be employed; the eye which is always used is more accustomed to the microscope, and the objects seen with it will appear more sharply defined than they will to the other eye. The eye thus used, however, if otherwise sound, becomes by degrees somewhat more short-sighted than the other. The eye which is not used is inactive for the time, even although it may not be shut. It is sometimes worth while to represent the upper and under-side of a section in one and the same drawing; in this case one of the sides is drawn first, and the outlines and important details of it are laid in with Indian ink, the primary pencil-marks are rubbed out, and the other side is then drawn over the first; the under-side must in this case be treated as if the observer were looking into the depth of the cells. Drawings of this sort, which, however, are seldom necessary, require some practice.

In representing the analysis of a flower, and in many other cases, it is often desirable to have a general drawing of the object as well as drawings of its details. A general drawing, if well executed, sets off the details. Here, also, if colour is not an object, it will be quite sufficient to have an outline drawing, provided it is rightly conceived. If much difficulty is met with in the execution of such a drawing, it is not worth while to

spend time over it, for accurate drawings of the details are always the most important, and must be primarily attended to. Too much cannot be done in this latter kind of drawing. Observation and drawing must be carried on simultaneously. The most beautiful general drawing is of little philosophical value without well-executed details to accompany it.

In following out the developement of the flower, or of the entire plant, it is often advantageous to delay the finishing of the drawings of particular parts; that is to say, it is advisable only to lay in the outline with a lead pencil, and to keep the specimen for some hours; during the progress of observations it will often happen that a better specimen than the first will be obtained, in which case the first outline may be rubbed out, and the better one substituted. By this means both time and paper are saved. In examining the developement of cells, on the other hand, the figure must be caught and fully represented at the earliest possible period; it is never safe to delay the completion of the drawing of cells, on account of the rapid and important changes which take place in them. In tracing the origin of the embryo of phanerogams, as accurate a drawing as possible should be made of both sides of the specimen whilst quite fresh, and another drawing should be made of the same specimen preserved in a solution of chloride of calcium. A comparison of these drawings will show the value of these preparations in the study of the impregnation of plants; it will be seen how little such preparations (especially of the plants Lathræa and Pedicularis) become changed in their principal features, and how fully they illustrate the questions with reference to which they were prepared.

Drawings of microscopical objects intended for publication should be etched upon stone. Any person who can draw tolerably well will find no difficulty in using the etching-needle, and when he can do this, he will himself, in cases of necessity, transfer his drawings to the stone, and thus be enabled to vouch for their accuracy with greater confidence. Every microscopical drawing must have marked upon it the magnifying power under which it was drawn. When the microscopical image is cast upon paper with the camera-lucida,

and at a measured distance from the latter, not only can the magnifying power be pretty accurately determined, but the relative size of the parts which have been drawn by the aid of the magnifying power can also be estimated: this may even be found out tolerably accurately with a pair of compasses.

In order to determine the magnifying power of any combination, the glass micrometer may be used. This is placed under the object-glass, and an image of it thrown, by means of the camera-lucida, upon a rule, or, what is better, the divisions of the micrometer are drawn upon paper, and transferred by the compasses to the rule. When, for instance, one-eighth of a millimetre (or 1-200th of an inch nearly) of the glass-micrometer covers 25 millimetres (or one inch nearly) of the rule, the magnifying power is eight times 25, or 200. By a similar easy mode of reckoning, all the magnifying powers may be determined, and a table prepared.

CHAPTER VIII.

ON THE PRESERVATION OF MICROSCOPICAL OBJECTS.

The preparation of lasting microscopical specimens is essential for the advancement of science. By means of them, difficult questions can often be most satisfactorily decided, and they are, moreover, of great use for the purpose of subsequent comparison with specimens afterwards obtained. It is only lately that any useful method of preparing such specimens has been discovered, and consequently it was not until lately that specimens of this nature were of any scientific value. The Royal Institute of the Netherlands, in the year 1847, propounded the question of the origin of the embryo as the subject of a prize essay, and required, that in addition to the manuscript and drawings, microscopical specimens should be furnished in support of the views deduced from observation, and represented in the drawings. In the adjudication of the Royal Institute, which awarded the prize to me, some praise was bestowed upon my specimens. I hope by means of those specimens, which are deposited at Amsterdam, and still more so, by later preparations from the Lathræa and Pedicularis which are in my own possession, that I have proved the origin of the first cell of the embryo in the interior of the pollen-tube, and that I have thereby established a most important point in vegetable physiology.*

The preservation of microscopical specimens can only be of value when the specimen itself is a good one; it is necessary, therefore, to be able to obtain specimens, and to form a judgment as to their value, before thinking of preserving them. I consider a collection of preserved specimens of things which can

* Upon this point see the note at p. 91.—Tr.

be procured in an equally good condition at any time, and without much labour, to be as valueless as a collection of bad specimens. A collection, on the other hand, of good specimens, prepared according to certain definite principles, is of great value and importance. I will first discuss the principles upon which such a collection should be prepared, and then proceed to the mode of preservation. A collection of microscopical specimens, if it is to afford aid towards the making of observations and the preparation of drawings, must be as complete as possible; that is to say, it must contain everything which is important for observation. In examining wood, for instance, the most beautiful transverse section, taken alone, is not sufficient; it is necessary that two equally good longitudinal sections (a radial and a tangential one) should also be preserved. In examining leaves the epidermis of both the upper and the under side must be taken, as well as thin transverse and longitudinal sections through the leaf. In tracing the developement of the embryo, &c., specimens of the object under examination in its different successive stages must be preserved.

As preservative media, I some time since selected three fluids.

1st. A solution of chloride of calcium. 2ndly. Glycerine. 3rdly. Copal varnish.

The solution of chloride of calcium is well adapted for all sections of wood and leaves, and is excellent for most species of tissue, even the youngest; preparations illustrating the impregnation of plants are the least changed by it, but the colouring matter in the cells is more or less injured. The grains of starch swell and become incapable of being recognized; but this seldom injures the general appearance of the specimen. This solution does not require to be hermetically confined. I have specimens of the most various kinds, prepared more than seven years since, which are not changed in the least. It is, therefore, highly to be recommended in cases in which a slight alteration in the colouring matter and in the grains of starch is not injurious. The solution should be mixed in the manner recommended at p. 9.

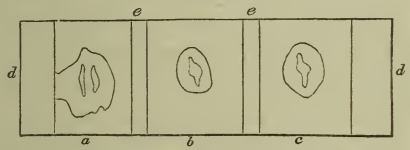
Glycerine is useful for the same objects, but the use of this fluid is of such recent date that I cannot answer for its preservative properties as confidently as for those of chloride of calcium. Specimens in glycerine, hermetically sealed, keep admirably. The chlorophyll and the grains of starch remain unchanged. The laminæ of the starch grains appear more beautiful after the lapse of twenty-four hours. Glycerine is, therefore, to be recommended when it is wished to preserve specimens containing chlorophyll or starch. In preserving delicate objects the glycerine should be previously diluted with water, since, when pure, it withdraws too much water from the young cell-membrane, and causes it to collapse.

Copal varnish and Canada balsam are useful for preserving less transparent objects, especially fossil woods; fresh sections of wood become too transparent in copal varnish, nevertheless it is as well, if many good specimens are at hand, to preserve some of them in copal varnish for comparison with other specimens preserved in chloride of calcium.

Schleiden, in his "Grundzüge," has treated fully of the method of preserving objects in fluid between two pieces of glass. I need, therefore, say very little about it, and will only refer to the principal points. The glass slides are made of a convenient size of thin, pure plate-glass, about one millimetre thick at the utmost; the glass need not be particularly white in colour, but it must be free from bubbles and from the débris of the material used for polishing it. These débris appear in the form of dark spots, generally of a red colour, upon the surface of the glass. The thinner the glass, the more agreeable it is to use, since then high powers can be employed. The length of the glass slides is regulated by the number of objects which it is wished to preserve on one of them, which objects are separated from one another by small strips of paper. I never preserve more than four objects, at the utmost, upon one slide. In specimens of wood I take, in addition to the three usual sections, some of the wood-cells and vessels which have been separated by maceration, and I place these cells and vessels in the fourth division on the same slide. The length of such a slide for four objects must not be less than eight centimetres,

the breadth must never be less than two centimetres, and it is better to make the slide rather wider. In fig. 50 is represented a glass slide with three objects on it; (*d*) and (*d*) are the wide strips of paper at the end of the slide; (*e*) (*e*) the smaller strips between the objects; (*a*) represents the manner in which the solution runs into the paper if too much of it be used; at *b* and *c* are seen drops of the proper size. An object is represented in the middle of each drop.

Fig. 50.



When the two glass slides have been cleaned with great care, the strips of paper are carefully fastened to one of them with a little gum; the strips at each end should be somewhat wider than those between the objects. These strips of paper serve not only to fasten the slides together afterwards, but principally to prevent pressure upon the objects. The paper which is chosen for the strips must therefore not be thinner than the object, since otherwise it would not fulfil these conditions. It must, however, not be very much thicker than the objects, because otherwise the latter would not lie securely, and might be injured by shifting. It is necessary, therefore, to be provided with paper of different thicknesses, and to be able to estimate the thickness of the objects to be mounted. In most cases, for instance, for sections of wood, only very thin foreign letter-paper can be used; in some few instances, as for objects illustrative of the question of developement, it is necessary to use stout drawing-paper. Objects of very unequal thickness cannot well be mounted on the same plate. The paper strips which divide the objects from one another are only useful in preventing the latter from being injured by accidental pressure. When the strips of paper are dry, and the slides once more well cleaned, a drop of solution of chloride of calcium is brought into the middle of each of the divisions by means of

a thin glass rod; it is a good plan first to breathe upon the slide; the drop then adheres better to the glass, it spreads out better, and there is less trouble in transferring the object. The objects must be first very carefully prepared; fresh specimens are seldom first treated with alcohol; sections of wood, on the other hand, must always be first placed in alcohol to drive out the resin and the air; they must not, however, be brought immediately from the alcohol into the solution; they must be placed in a watch-glass with water, in order that the alcohol may be driven out of them. Each particular object is now very carefully taken up with a very fine camel-hair brush, and is brought into the drop of the solution intended for it; it will often be a good plan to place the watch-glass on a dark object, such as blackened wood or paper; the small objects are then found with greater facility. When the objects are all brought upon the slide, the latter is placed under the simple microscope, and the objects are brought into the right position by carefully spreading them out with a needle; at the same time the small particles of dust, as well as the threads or hairs which are always met with, and which have accumulated during the preparation of the object, must be removed. In transferring the objects with the brush from the water to the solution, it is impossible to avoid diluting the latter; it is, therefore, advisable to remove the greater part of the fluid in which the object lies with a very clean brush of a coarser kind. With a little practice, this may be done without touching or disturbing the object. The fluid which is removed is then replaced by a fresh drop of the solution. The size of this drop is regulated by the thickness of the strips of paper which are used: if the drop has become too large, a portion of the fluid should be removed, as before, with the brush. Before fastening on the covering-glass, it is advisable to place the object under the simple microscope, or under the compound microscope with a low power, in order, if necessary, to ascertain whether the objects can be improved. The whole of the paper strips on the lower glass are now smeared over carefully with a little gum, the upper glass is placed carefully upon it, and the two are pressed closely together with the thumbs of both hands. This pressure must not be

applied beyond the limits of the divisions, since otherwise the objects are apt to be injured. The ends of the slides are afterwards fastened with thin paper, upon which any remarks relative to the objects, such as the date, &c., may be noted.

The principal difficulty in mounting objects in this way consists in taking the right quantity of the solution; if too little is used, the object will not be sufficiently immersed, if too much, the fluid is attracted into the paper divisions, and is drawn away from the object; the object is not spoilt, because it is still saturated with the solution, but it is not so good for the purposes of observation, and it often becomes necessary in such a case to detach the glass plates from one another by soaking them in water, and to mount the objects afresh. If the object is placed, as it should be, in the middle of a completely isolated drop of the solution in the manner represented in fig. 50, *b*, *c*, there is no further fear as to its preservation.

When glycerine is employed the same mode of proceeding may be adopted, but in this case the joinings of the glass plates are fastened together with Canada balsam or copal varnish, as soon as the gum at the divisions has become dry. There is always some difficulty in hermetically closing the sides of the plates; it is always doubtful how far the closing is perfect; it is therefore necessary to spread the copal varnish several times over the joinings, waiting each time for the previous coating to dry before applying a fresh one. I am, unfortunately, but little acquainted with the use of glycerine in preserving microscopical objects; as it is not capable of fermentation, it is, perhaps, unnecessary that it should be hermetically sealed; but it is requisite that further experiments should be made with regard to its properties; if it need not be hermetically sealed, glycerine will be a very important preservative medium.

In using copal varnish, the glass plates must be treated in a similar manner, and, in addition, it will be advisable to warm the bottom plate, and to transfer the objects from alcohol or ether into the copal varnish. The warming expels all the moisture from the object, and at the same time the varnish is thickened by the evaporation of the oil of turpentine. The covering-glass is then warmed, and fastened to the lower one.

I have found in practice that it is useless to attempt to close up objects hermetically by smearing them with melted caoutchouc. All the objects which I have set up in this way, although mounted with great care, have been spoilt. The melted caoutchouc appears to be very sensible of the least change of temperature, it insinuates itself here and there between the plates, by which means, not only does the closing cease to be hermetical, but the object itself is pushed about from place to place, or becomes embedded in a mass of caoutchouc. The disadvantages of this method of mounting do not always show themselves in the first few weeks, or even months, but there are very few cases in which they do not eventually become apparent. Preservation in glycerine affords the same advantages with respect to the brightness of the object, as eau sucré, &c.

In keeping the object-slides, it is only necessary to take care that they always lie flat, because, if they are placed upon their edges for any time, the solution is easily drawn away from the middle: the mutual pressure of several slides lying upon one another seldom does any harm; the slips of paper protect the objects. In any large collection it is advisable to arrange the objects, either according to the nature of the plants, or of the parts which are preserved.

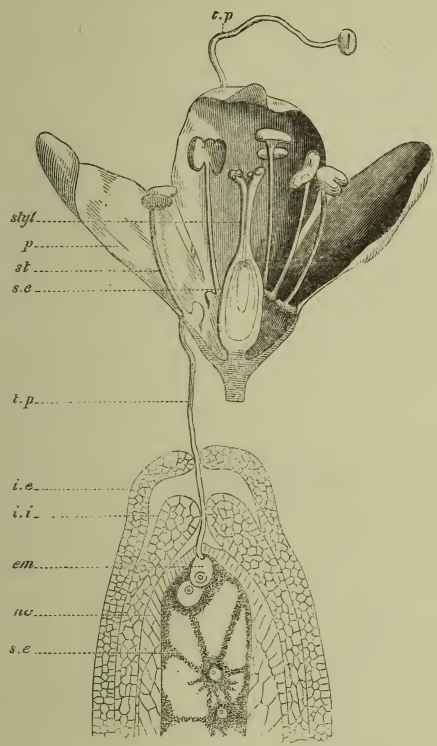
APPENDIX.

The following is Unger's account of the origin of the embryo, taken from the Twelth Letter of his *Botanische Briefe*. After describing the formation of the ovule, he proceeds as follows :—

"Shortly afterwards, there is found in the centre of the ovule a cell, perhaps the top cell, which grows to a very large size ; it is called the embryo-sac, and in its spacious interior several small free cells are produced, which float about in the contents of the former cell. These are, and can be, no other than reproductive cells. These reproductive cells do not appear of themselves to increase any further in growth, and they would wither and eventually decay if they were not moved to further developement by external agency. The same would be the case with the free pollen-grains. It comes to pass, however, that the free pollen-grains and the cells of the embryo-sac come together, and, whilst the former perish by the contact, there arises in the latter a capacity for further developement; the result of which is, that the whole ovule, now called *the seed*, becomes detached from the mother-plant, and the young plant, already formed, is placed in a condition to carry out, unaided, its own developement. This young plant naturally follows in every respect the type of the mother-plant. The reproductive cells of the ovule are undoubtedly enclosed within it, the ovule itself being generally situated in the hollow of the united carpels, that is, hidden in the ovary; but this does not prevent the free pollen-grains from coming into immediate contact with the reproductive cells of the ovule. This takes place in the following manner : the position of the ovary is such, that amongst the many thousand pollen-grains which become free after the dehiscence of the anther,

some must necessarily come in contact with it, and especially with its apex. This apex, called the style, which originates in the growing together of the apices of the carpels, and which, according to the form of the latter, is shorter or more elongated, spreads out somewhat more widely at the point called the stigma. Those pollen-grains which fall upon the stigma are acted upon by a moist secretion, continually exuding from the stigma, which superinduces a further developement, a further growth, or, as one may say, a *germination*, the result of which is, that a cellular utricle is developed behind the external covering of the pollen-grain, which utricle, although single, is in a condition to branch out, by protruding itself through the external covering. The germinating pollen-grain, however, notwithstanding the moisture of the stigma, would soon perish if it were not in a condition, by means of its projecting end, to make a passage for itself between the loose cells of the stigma, and between the slightly-connected tissue of the style. After some time one or more of the pollen-grains always succeeds in effecting a passage downwards into the ovary. There are now but few more difficulties to be overcome. The apex of the growing pollen-tube easily reaches the ovule, and finds there, through the openings in the coats of the ovule, an uninterrupted passage to the nucleus. Finally, however, the cells of the nucleus must be broken through. This is easily done, inasmuch as these cells are still very tender and yielding, and, at the same time, the embryo-sac, through its own extension and the displacement of the cells above, is, to a certain extent, brought in apposition to the pollen-tube. The germ-cells in the interior of the embryo-sac itself are found at this period near the surface; they even touch the inner side of its wall. It is, therefore, an easy matter for the pollen-tube, having penetrated thus far, to come into immediate contact with the germ-cells, from which it is only separated by the membrane of the embryo-sac; the pollen-tube even spreads itself over the surface of the embryo-sac in order, if possible, forcibly to bring about this contact. The result is, that whilst the pollen-tube withers by degrees, the process of decay being from the exterior inwards, a further cell-formation commences in one of the germ-cells, probably in that one which lies next to the pollen-tube, which cell-formation terminates in the production of the rudiments of a new plant. It appears not improbable that in many cases the embryo-sac becomes completely absorbed at the point of contact of the pollen-tube, so as to enable the pollen-tube and germ-cells to come into immediate contact with one another; but this is not yet sufficiently proved by experience."

The annexed figure is given by Unger in illustration of the above views. It represents a magnified longitudinal section of the flower



of Fagopyrum emarginatum. (*p.*) is the perianth ; (*st.*) the stamens, the anthers having already burst. Some of the pollen-grains are shown in contact with the stigma. They have already become elongated, and have penetrated through the canal of the style down to the embryo-sac. By magnifying the upper part of the ovule more highly, as in the lower figure, the whole course of the pollen-tube (*t.p.*) may be traced successively through the micropyle, the integumentum externum (*i.e.*), the integumentum internum (*i.i.*), and the nucleus (*nc.*), down to the embryo-sac (*s.e.*), where it comes into immediate contact with the germ-cells (*e.m.*)

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